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# THE ROLE OF PHOSPHOGLYCERIC ACID IN THE DISSIMILATION OF GLUCOSE BY BACTERIA OF THE ESCHERICHIA-AEROBACTER GROUP

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In 1933 Embden, Deuticke, and Kraft upset existing theories of muscle glucolysis by proposing the following initial stages:

(1) glucose + $2H_3PO_4$ + hexose diphosphate → 4 triose phosphate → 2 phosphoglyceric acid + 2 α-glycerophosphoric acid.

This scheme has been confirmed and somewhat modified by Meyerhof and Kiessling (1933), and applied in principle to alcoholic fermentation (1934). The same authors (1935) have presented evidence that methylglyoxal is not an intermediate in glucose dissimilation but arises as a stabilization product from an intermediate triose. In the light of the findings on muscle and yeast glucolysis, it is of interest to reinvestigate the mechanism of bacterial dissimilation, particularly as present schemes generally accept methylglyoxal as the key intermediate.

Recently Tikka (1935) has suggested that the initial steps of glucose breakdown by *Escherichia coli* may follow the same path as Embden's scheme for muscle. Although Tikka showed that fresh living cells of *Esch. coli* dissimilated phosphoglyceric acid and α-glycerophosphate, he was unable to isolate either compound. Werkman, Zoellner, Gilman and Reynolds (1935) have already reported the isolation of phosphoglyceric acid from glucose by resting cells of *Citrobacter freundii*. The isolation of phosphoglyceric acid from the dissimilation of glucose by *Esch. coli* and *Aerobacter indologenes* is reported in this communication. The evidence suggests strongly that phosphoglyceric acid plays an essential role in bacterial dissimilation, corresponding to its function in muscle and yeast glucolysis.

## EXPERIMENTAL

The isolation of the phosphoglyceric acid formed by *Escherichia coli* was accomplished by the method of Neuberg and Kobel (1933) with certain modification. In a small flask were placed 17 gm. fresh cell paste (prepared by super-centrifuging the cells from a glucose-peptone broth), 25 cc. 0.67 M phosphate buffer (pH 6.8), 32 cc. 20 per cent glucose, 0.5 cc. 1 per cent $MgCl_2$, 5 cc. $H_2O$ and 3 cc. toluol. The mixture was warmed to 37° C., 0.5 cc. removed for phosphate determination and the flask placed in a 37° C. incubator. The initial or phosphorylation period was terminated after 3 hours; 25 cc. of 2 per cent acetaldehyde and 4 cc. of 0.2 M sodium fluoride were added. Again a 0.5 cc. sample was taken for phosphate determination and the mixture returned to the incubator. The second period was completed in 3.5 hours and the mixture was centrifuged to remove the cells. The inorganic phosphate was precipitated by

addition of 12 cc. of 20 per cent Mg acetate to the solution, and made alkaline with $NH_4OH$. Ten cc. of glacial acetic acid and 5 cc. of 50 per cent Ba acetate were added to the filtrate. The resulting precipitate was immediately removed by centrifuging and from the supernatant liquid 0.226 gm. of the barium salt of phosphoglyceric acid slowly crystallized after 36 hours at 5° C. The phosphate determinations were made colorimetrically by the method of Kuttner and Lichtenstein (1930). The results are given in the accompanying table.

For the isolation of phosphoglyceric acid from *Aerobacter indologenes*, substantially the same procedure was used. Only 20 cc. of 20 per cent glucose were added and the initial mixture made up to 60 cc. At the end of the first period 15 cc. of 2 per cent aceteldehyde and 2 cc. of 0.2 M NaF were added and 0.257 gm. of the crude phosphoglyceric acid salt was obtained. It is important to point out that although the procedures given above do not necessarily give the optimal yield of the ester, small variations in the method may produce large variations in yield, making the preparation difficult and uncertain.

*Determination of inorganic phosphate in reaction mixtures*
(calculated as mgms. P/cc.)

| Trial | Organism | Phosphate in reaction mixture | | | Phosphate uptake | | |
|---|---|---|---|---|---|---|---|
| | | Original | End of first period | Final | First period | Second period | Total |
| 54 | *Esch. coli* | 5.38* | 4.33 | 3.52 | 1.05 | 0.81 | 1.86 |
| 49 | *A. indologenes* | 5.97* | 4.73 | 5.03 | 1.24 | —0.30 | 0.94 |

* Corrected for dilution occurring by addition of acetaldehyde and fluoride solutions.

The crude phosphoglyceric acid salt was purified by solution in seventy times its weight of 0.05N HCl and reprecipitated by addition of two volumes of 95 per cent ethyl alcohol. The barium salt appeared as small white leaflets, giving the mixture a translucent sheen. The optical rotation of a 2.7 per cent solution is $-0.35°$ ($[\alpha]^{28°}_{D} = -13.0$). Neuberg obtained $-0.36°$ for phosphoglyceric acid from yeast. The behavior of the compound was observed to be identical with that of the barium salt obtained from Citrobacter, Propionibacterium, and yeast.

The mechanism of formation of the phosphoglyceric acid may be represented as follows. During the first period phosphorylation of the glucose occurs accounting

(2) glucose $+ 2H_3PO_4 \rightarrow$ hexose diphosphate

for the phosphate uptake shown in the initial period. Meyerhof and Kiessling (1934) believe that in alcoholic fermentation the presence of the hexose diphosphate is necessary for the breakdown of glucose to proceed

at a normal rate. After formation of the hexose ester, presumably some phosphoglyceric acid may be formed by reaction 1. However, as there is no fluoride present, the phosphoglyceric acid formed is broken down. It is evident that as soon as sufficient hexose diphosphate is produced, the first period should be ended and the sodium fluoride and acetaldehyde added. In the presence of acealdehyde and hexose diphosphate the main reaction for the formation of the ester is probably

(3) glucose $+ 2H_3PO_4 + 2\ CH_3CHO \rightarrow 2$ triose phosphate $+ 2\ CH_3CHO \rightarrow 2$ phosphoglyceric acid $+ 2\ CH_3CH_2OH$.

Such a reaction will result in a phosphorus uptake for the second period as shown in the table for trial 54 with *Esch. coli*. However, *A. indologenes* shows a decrease in inorganic phosphate during the second period. Apparently the dephosphorylation of phosphate esters formed in the first period proceeded more rapidly than the reaction given above.

It may be objected that the isolation of phosphoglyceric acid from the toluol treated organisms does not give us a true picture of an unpoisoned normal fermentation. This objection must be considered because at the present time the exact function of the toluol is unknown. Neuberg and Kobel explain its action as that of a cell plasmolyzing agent. It may be safely said that the organisms possess the necessary enzyme equipment for the production of phosphoglyceric acid. The isolation, coupled with the fact that Tikka has shown *E. coli* able to effect its breakdown to normal end products, presents strong evidence that phosphoglyceric acid is a normal intermediate in fermentation by Escherichia and Aerobacter. It is apparent that the mass of evidence for the Embden-Meyerhof scheme of muscle glycolysis may equally well apply to bacterial dissimilation.

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# OXIDATIVE DEGRADATION OF SILK[1]

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Silk is exposed to oxidants of the atmosphere and certain processes of bleaching, dyeing, and printing. A review of the literature has disclosed a great number of empirical recipes for bleaching silk with permanganates, peroxides, perborates, percarbonates, and persulfates but no quantitative information about the effect of these oxidants on its composition and physical properties. Wild silk fibroin has been characterized as more resistant than silk fibroin to oxidizing mordants (13); three-volume as well as ten-volume hydrogen peroxide has been recommended for bleaching (17, 10), though described as yellowing wild silk at a high temperature (2); ozone has been found to make silk yellow, harsh, and lusterless (4); and the use of sodium perborate, recommended for the oxidation of Indanthrene dyeings on silk (21), has been considered hazardous for weighted silk (16). The effects of weathering and storage on the composition and mechanical performance of silk have been investigated but without separation of the factors involved. The effectiveness of reductants in retarding degradation of weighted silks ascribed to oxidation has been questioned (15, 24, 23, 9, 3, 20).

Quantitative data of the effect of hydrogen peroxide and aqueous potassium permanganate in ten hours at 40° C. on the weight, nitrogen, ash, and wet strength of wild silk fibroin, silk fibroin, black iron-weighted, white lead-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silks of typical commercial quality are reported in this study.

## EXPERIMENTAL PROCEDURE

### PREPARATION OF FABRICS

Silk crêpe, plain-woven in the gum, was boiled one hour in one hundred volumes of ten per cent neutral olive-oil soap, rinsed, boiled in another bath of the soap and then in water three times for 15 minutes each. Plain-woven pongee of wild silk was boiled one hour in water, rinsed, and again boiled in water for 15 minutes, and thoroughly rinsed. These fabrics were cut for analysis, continuously extracted with anhydrous ether for 18 hours and, with the exception of samples for physical analysis, dried at 105° to 110° C. until successive weighings with tare checked within half a milligram. The plain-woven weighted silks were cut for analysis or dried to constant weight without any pretreatment.

### ANALYSIS OF FABRICS

The seven fabrics were conditioned for four or more hours at 70° ± 2° F. and 65 ± 2 per cent R. H. before analysis for weight, thick-

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[2] The authors wish to thank Miss Florence Barr for checking some of the analyses.

TABLE 1. *Analysis of the silk fabrics*

| Silk | Weight | Thickness | Yarn | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Number per inch | | Percentage of fabric | | Count | | Twist* |
| | | | | | | | *typp* | | *number per inch* |
| | *oz. per sq. yard* | *inch* | *warp* | *filling* | *warp* | *filling* | *warp* | *filling* | *filling* |
| A. Iron-weighted silk crêpe | 3.00 | 0.0066 | 160 | 74 | 69.0 | 31.1 | 48.7 | 46.9 | 62 (2)** |
| B. Lead-weighted silk crêpe | 3.17 | 0.0065 | 127 | 74 | 60.6 | 37.8 | 37.4 | 35.3 | 31 (2) |
| C. Tin-weighted silk crêpe | 2.86 | 0.0063 | 155 | 72 | 65.1 | 34.9 | 44.8 | 44.8 | 75 (5) |
| D. Tin-lead-weighted silk crêpe | 3.15 | 0.0065 | 180 | 79 | 71.4 | 28.5 | 45.4 | 48.5 | 84 (4) |
| E. Zinc-weighted silk crêpe | 2.86 | 0.0062 | 170 | 70 | 73.0 | 26.0 | 51.9 | 48.5 | 62 (3) |
| F. Silk crêpe | 2.20 | 0.0067 | 246 | 109 | 59.0 | 40.3 | 127.8 | 88.2 | 60 (2) |
| G. Wild silk pongee | 1.25 | 0.0042 | 76 | 77 | 45.3 | 53.7 | 71.7 | 78.0 | 0 |

* Two left-twisted filling yarns alternated with two right-twisted filling yarns; the warp yarns were not measurably twisted.

** Average deviations.

TABLE 1. (Continued)

| Silk | Ash | | Breaking strength | | | | Elongation at breaking load | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Of conditioned fabrics | | Of wet fabrics | | Of conditioned fabrics | | Of wet fabrics | |
| | *percentage of yarn* | | *pounds per inch* | | *percentage of dry* | | *percentage* | | | |
| | *warp* | *filling* | *warp* | *filling* | *warp* | *filling* | *warp* | *filling* | *warp* | *filling* |
| A. | 42.5 | 40.2 | 34 (0.6)** | 12 (0.2)** | 97 | 75 | 10 | 7 | 25 | 34 |
| B. | 44.5 | 44.6 | 30 (0.3) | 12 (0.7) | 57 | 58 | 7 | 7 | 16 | 23 |
| C. | 54.2 | 52.9 | 38 (1.0) | 13 (0.5) | 53 | 46 | 10 | 9 | 35 | 23 |
| D. | 52.6 | 48.7 | 42 (0.3) | 13 (0.5) | 57 | 46 | 10 | 7 | 35 | 28 |
| E. | 53.7 | 49.9 | 40 (0.4) | 14 (0.4) | 58 | 50 | 10 | 8 | 35 | 31 |
| F. | | | 38 (0.9) | 32 (0.4) | 92 | 81 | 34 | 37 | 56 | 55 |
| G. | | | 21 (0.4) | 23 (1.3) | 81 | 43 | 30 | 30 | 38 | 37 |

TABLE 1. (Continued)

| Silk | Aluminum | Ash | Iron | Lead | Nitrogen | Phosphorus | Silica | Tin | Water extract | Weighting | Zinc |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *percentage of fabric* | | | | | | | | | | |
| A. | | 43.7 | 15.11 | | 8.16 | 0.56 | 12.52 | | 10.9 | 70.6 | |
| B. | trace | 44.2 | | 12.21 | 9.17 | 0.91 | 20.30 | | 8.8 | 55.3 | |
| C. | | 52.9 | | | 9.26 | 0.45 | 20.49 | 8.80 | 7.2 | 61.3 | |
| D. | trace | 50.3 | | 9.10 | 8.46 | 1.06 | 14.91 | 8.66 | 4.4 | 60.8 | |
| E. | 0.85 | 51.9 | | | 8.10 | 0.57 | 15.07 | | 4.6 | 63.5 | 11.12 |
| F. | | 0.3 | | | 18.59 | | | | | | |
| G. | | 0.6 | | | 18.25 | | | | | | |

ness, yarns, breaking strength by the one-inch-strip method, and elongation at breaking load (1, 5).

The nitrogen of four-gram samples of silk fibroin or wild silk fibroin was determined by the Kjeldahl-Gunning-Arnold method (11), that of the weighted silks by the Kjeldahl method (14).

Each value for water extract and weighting is the average of four determinations made with five-gram samples (25). Two four-gram samples of yarn were dried to constant weight at 105° C. and ignited until constant in an electric furnace at dull red heat; the ash of the iron-weighted silk was treated with nitric acid and again ignited. For each fabric four five-gram samples were similarly analyzed for total ash. The phosphorus of the ash was determined gravimetrically (6A) and the silica as loss in weight upon treatment with hydrofluoric acid (8). The residues from hydrofluoric-acid treatment of the ash were then further analyzed; that of the iron-weighted silk was fused with potassium pyrosulfate before solution in dilute sulfuric acid for volumetric determination of iron (22b); the lead of the lead-weighted and tin-lead-weighted silks was weighed as sulfate (22c) and the tin of the tin-weighted and tin-lead-weighted silks as stannic oxide (6b); the aluminum of the zinc-weighted silk was weighed as oxide (22a) and the zinc was determined volumetrically (22d). Analyses were made with separate samples except in cases of tin and lead of the tin-lead-weighted and aluminum and zinc of the zinc-weighted silks.

Throughout this study the average of three or more chemical determinations has been expressed as percentage of textile dried to constant weight at 105° to 110° C. and weighed with tares. The fabrics are described by analysis in table 1.

### TREATMENT OF FABRICS WITH HYDROGEN PEROXIDE

Four grams of silk fibroin or wild silk fibroin were immersed in 200 cc. of 0.005 *M* sodium caronate, 1.0900 *M* or 2.1800 *M* hydrogen peroxide (27) made 0.005 *M* with respect to sodium carbonate, in a 250-cc. stoppered Erlenmeyer flask in a water bath at 40° ± 0.1° C. for ten hours and then washed in water until the rinse no longer reduced permanganate. After drying at room temperature the residual silks were analyzed for nitrogen or again dried to constant weight.

TABLE 2. *Effect of hydrogen peroxide in ten hours at 40° C. on the weight, nitrogen, and wet strength of silk fibroin and wild silk fibroin*

| Hydrogen peroxide | Sodium carbonate | Weight | | Nitrogen | | Breaking strength of wet warp | |
|---|---|---|---|---|---|---|---|
| | | Silk | Wild silk | Silk | Wild silk | Silk | Wild silk |
| *molality* | *molality* | *percentage of original fabric* | | | | *pounds per inch* | |
| 0 | 0.005 | 99.8 | 99.0 | 18.61 | 18.16 | 26 | 16 |
| 1.0900 | 0.005 | 96.9 | 97.6 | 18.06 | 17.95 | 17 | 12 |
| 2.1800 | 0.005 | 93.3 | 96.3 | 17.10 | 17.65 | < 1 | 11 |

Ten warp strips for test of strength, treated in the same way but without initial drying, were tested wet after rinsing. The weighted silks were

unchanged in wet strength after exposure to high concentrations of hydrogen peroxide for ten hours at 40° C., although the peroxide was decomposed by the weighting (12).

### TREATMENT OF FABRICS WITH AQUEOUS POSTASSIUM PERMANGANATE

Approximately four grams of wild silk fibroin were immersed in 200, 500 or 1000 cc. of 0.0237 *M* potassium permanganate (7) ten hours at 40° ± 0.1° C., freed of manganese dioxide in two hours by 0.05 *M* sodium hydrogen sulfite, and washed in water until the rinse failed to reduce permanganate. After drying at room temperature the residues were either analyzed for nitrogen or dried to constant weight. Because of decrease in weight and linear decrease in nitrogen of wild silk fibroin with increasing volume of permanganate (table 3), fifty volumes of permanganate per gram of fabric were used in further treatments.

TABLE 3. *Effect of volume of aqueous potassium permanganate on the weight and nitrogen of wild silk fibroin in ten hours at 40° C.*

| Potassium permanganate | | Weight | Nitrogen |
|---|---|---|---|
| *cc. 0.0237 M* | *gram per gram fibroin* | *percentage of original fabric* | |
| (200 cc. water) | 0 | 99.5 | 18.24 |
| 200 | 0.466 | 96.4 | 17.86 |
| | 0.493 | 96.6 | 17.86 |
| | 0.512 | 96.5 | 17.77 |
| | 0.572 | 96.0 | 17.76 |
| 500 | 1.343 | 94.0 | 17.13 |
| | 1.447 | 92.7 | 17.02 |
| 1000 | 2.358 | 83.8 | 15.73 |
| | 2.401 | 83.0 | 15.51 |

In the case of each of the fabrics approximately five grams were immersed for ten hours at 40° ± 0.1° C. in fifty volumes of water or permanganate in a 500-cc. stoppered Erlenmeyer flask. The residual fabric was freed of manganese dioxide in 30 minutes by fifty volumes of 0.05 *M* sodium hydrogen sulfite and washed in water until the rinse did not reduce permanganate; sodium hydrogen sulfite under these conditions had no effect on silk fibroin or wild silk fibroin. Wet warp strength was determined at once, nitrogen after drying at room temperature, and ash after drying to constant weight.

## DISCUSSION OF RESULTS

One factor of the greater degradation of the silk fibroins by permanganate (tables 5 and 7) than by peroxide (table 2) is the dissipation of the alkaline hydrogen peroxide by rapid evolution of oxygen (blank determinations testing 1.0900 *M* and 2.1800 *M* decreased to 0.2463 *M* and 0.7666 *M*, respectively, in ten hours at 40° C.); another and probably a large factor is the equimolal concentration of potassium hydroxide resulting from complete decomposition of the permanganate (26, 19).

The weight and ash of the residual weighted silks (table 4) show that little weighting was removed by the permanganate.

TABLE 4. *Effect of fifty volumes of aqueous potassium permanganate in ten hours at 40° C. on the weight, ash, and nitrogen of the weighted silks*

| Potassium permanganate | Iron-weighted silk | | | Lead-weighted silk | | | Tin-weighted silk | | |
|---|---|---|---|---|---|---|---|---|---|
| *molality* | Weight | Ash | Nitrogen | Weight | Ash | Nitrogen | Weight | Ash | Nitrogen |
| | *percentage of original fabric* | | | | | | | | |
| 0 | 96.5 | 40.2 | 8.08 | 93.0 | 44.1 | 9.08 | 93.1 | 51.4 | 8.64 |
| 0.0030 | 96.0 | 40.2 | 8.02 | 92.9 | 40.9 | 8.94 | 92.2 | 51.2 | 8.46 |
| 0.0060 | 95.0 | 39.6 | 7.47 | 88.7 | 40.1 | 8.26 | 92.0 | 50.0 | 7.70 |

TABLE 4. (Continued)

| Potassium permanganate | Tin-lead-weighted silk | | | Zinc-weighted silk | | |
|---|---|---|---|---|---|---|
| | Weight | Ash | Nitrogen | Weight | Ash | Nitrogen |
| *molality* | *percentage of original fabric* | | | | | |
| 0 | 96.0 | 49.0 | 8.50 | 96.0 | 50.1 | 7.79 |
| 0.0030 | 95.7 | 48.6 | 8.36 | 94.0 | 50.0 | 7.39 |
| 0.0060 | 94.7 | 48.4 | 7.66 | 89.4 | 49.5 | 6.85 |

TABLE 5. *Effect of fifty volumes of aqueous potassium permanganate in ten hours at 40° C. on the weight, ash, and nitrogen of silk fibroin and wild silk fibroin*

| Potassium permanganate | Silk fibroin | | | Wild silk fibroin | | |
|---|---|---|---|---|---|---|
| | Weight | Ash | Nitrogen | Weight | Ash | Nitrogen |
| *molality* | *percentage of original fabric* | | | | | |
| 0 | 99.4 | 0.3 | 18.56 | 99.5 | 0.7 | 18.25 |
| 0.0030 | 98.7 | 0.5 | 18.21 | 99.2 | 0.7 | ..... |
| 0.0060 | 98.7 | 0.6 | 18.22 | 99.1 | 0.8 | 18.23 |
| 0.0083 | 98.6 | 0.7 | 18.11 | 98.4 | 1.1 | 18.12 |
| 0.0167 | 97.9 | 0.8 | 17.72 | 98.4 | 1.3 | 17.90 |
| 0.0237 | 97.7 | 1.0 | 17.68 | 97.9 | 1.4 | 17.81 |

TABLE 6. *Percentage of fibroin* dissolved in ten hours at 40° C. by fifty volumes of aqueous potassium permanganate*

| Potassium permanganate | Fabric | | | | | | |
|---|---|---|---|---|---|---|---|
| *gram per gram fibroin* | A | B | C | D | E | F | G |
| 0.0969 | 6.0 | *9.0* | 10.0 | 8.7 | 9.3 | 3.4 | 1.3 |
| 0.1017 | 6.6 | | *10.9* | 9.5 | 10.1 | 3.5 | 1.4 |
| 0.1035 | 6.9 | | | *9.8* | 10.5 | 3.6 | 1.4 |
| 0.1090 | *7.6* | | | | 11.4 | 3.8 | 1.5 |
| 0.1129 | | | | | *12.1* | 3.9 | 1.6 |

* Calculated from nitrogen; values in italics are experimental, the others interpolated.

TABLE 7. *Effect of fifty volumes of aqueous potassium permanganate in ten hours at 40° C. on the wet warp breaking strength of the fabrics*

| Potassium permanganate | Fabric | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G |
| *molality* | *pounds per inch* | | | | | | |
| 0.0000 | 32 | 20 | 24 | 21 | 21 | 27 | 15 |
| 0.0030 | 30 | 10 | 17 | 9 | 17 | 12 | 6 |
| 0.0060 | 23 | 9 | <1 | 6 | <1 | 10 | 4 |
| 0.0083 | <1 | <1 | | <1 | | 9 | 3 |
| 0.0167 | | | | | | 6 | 2 |
| 0.0237 | | | | | | <1 | <1 |

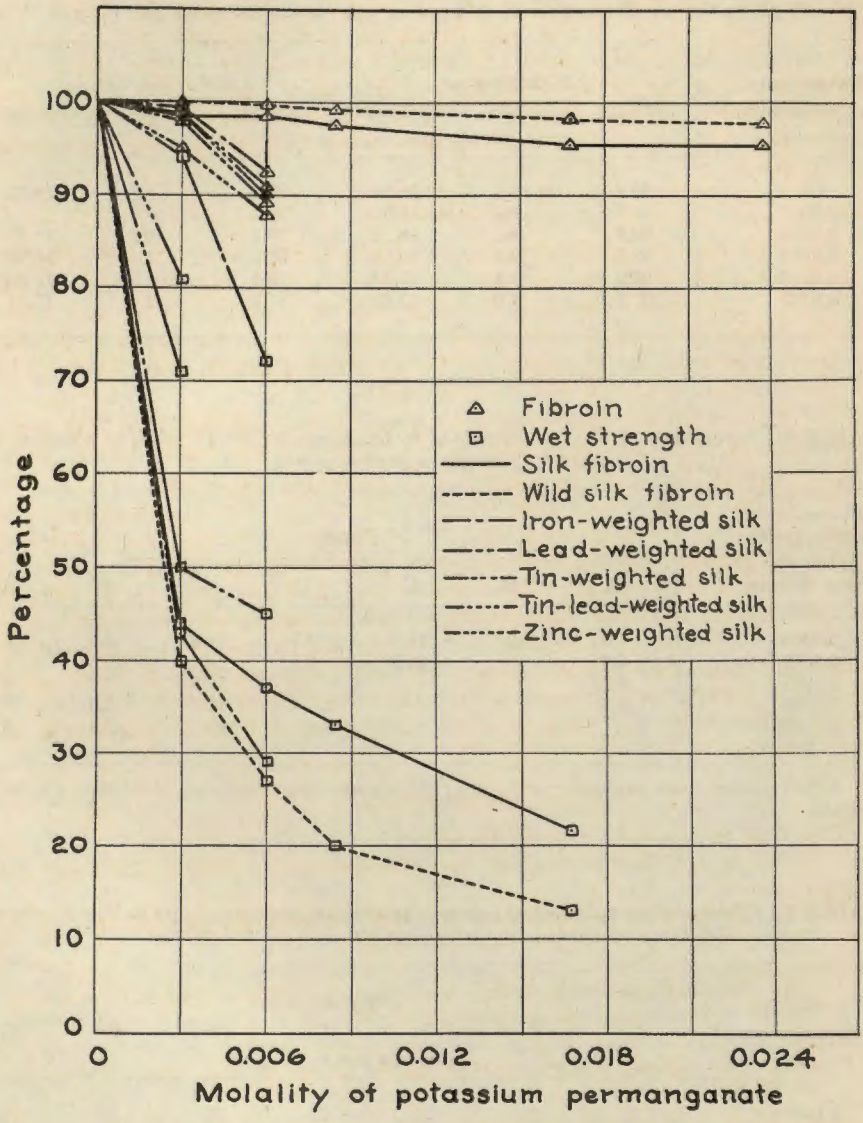


Fig. 1. Effect of fifty volumes of aqueous potassium permanganate in ten hours at 40° C. on the fibroin and wet strength of the fabrics.

Although dilution of fibroin by weighting increases its oxidative degradation to soluble forms of nitrogen (tables 4, 5 and 6), the loss and rate of loss in wet strength with increasing concentration of oxidant exceed the loss and rate of loss of fibroin (Fig. 1). The pattern of the mechanical performance of the iron-weighted, lead-weighted, tin-weighted, and zinc-weighted silks during oxidative degradation shows their wet strengths as high or higher than that of silk up to the time of their complete degradation. This rapid loss of strength is similar to that which occurs in dry cleaning and laundering lead-weighted and tin-lead-weighted silks (18).

## SUMMARY

1. The course of the degradation of silk fibroin and wild silk fibroin by hydrogen peroxide and of silk fibroin, wild silk fibroin, black iron-weighted, white lead-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silks by aqueous potassium permanganate in ten hours at 40° C. has been followed by analysis of the residual plain-woven fabrics for weight, nitrogen, ash, and wet warp strength.

2. The weighted silks were unchanged in wet strength by high concentrations of hydrogen peroxide; wild silk fibroin has been shown more stable than silk fibroin to 1.09 and 2.18 *M* hydrogen peroxide.

3. It has been shown that degradation of the fabrics by potassium permanganate is much greater than by hydrogen peroxide; that nitrogen of wild silk fibroin is a decreasing linear function of the volume of potassium permanganate at a given concentration; that nitrogen of silk fibroin and nitrogen of wild silk fibroin are almost linear functions of the concentration of oxidant; that dilution of fibroin by weighting results in greater solution of its nitrogen upon oxidative degradation; and that loss and rate of loss in wet strength with increasing concentration of permanganate, different for the various fabrics, are greater than loss and rate of loss of fibroin.

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# DEGRADATION OF FIVE WEIGHTED SILK FIBROINS BY STEAM[1]

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Accepted for publication July 14, 1936

A recent editorial traces the tremendous annual losses in the drying of textiles to the blind acceptance of meaningless "safe drying temperature limits," regardless of the textile, its regain or chemical processing, and time of steaming (12). Although steam is used in lustering (9), conditioning (11), and aging dyed and printed weighted silks (1, 2, 3, 4, 5, 8, 10) as well as in their maintenance during use, there is little description of the effect of steam on the composition and properties of weighted silks.

The 48 per cent loss of dry strength upon steaming a silk 46 hours at 100° C. and the 55 per cent loss of this same silk when tin-weighted, compared to corresponding losses of 25 and 22 per cent upon drying the silks the same time at the same temperature, suggest that weighting and its hydrolytic products are factors of the greater deterioration by steam (6).

We have measured the relative resistance to degradation by steam of plain-woven crêpes of silk fibroin, iron-weighted, lead-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silk fibroins by analysis of the residual fabrics for weight, ash, nitrogen, and wet strength.

## EXPERIMENTAL PROCEDURE

Five four-gram samples of one of the fabrics (previously defined analytically 7), or five one-inch-warp strips were steamed at one time in an autoclave equipped with an accurate pressure gauge and thermometer; the fabrics, attached by silk thread to glass rods laid across the top, were hung in a four-liter Pyrex beaker having a low exit for steam and were protected by an inverted watch glass from any liquid which had flowed across metal. In one series of tests the fabrics were steamed at 0, 8, 18, 28, 33, and 38 pounds for one hour; in another series steaming at eight pounds was continued for two, three, four, and five hours. The residual fabric was rinsed in water eight times to remove soluble derivatives; wet strength was determined at once, nitrogen after drying at room temperature, and ash after drying the weighted silks to constant weight (7). Each value reported for wet strength is the average of ten determinations; values for weight, ash, and nitrogen are the averages of four closely agreeing determinations expressed as percentage of the original fabric dried at 105° to 110° C. until successive weighings with tare checked within half a milligram.

## DISCUSSION OF RESULTS

The greater degradation of the silk fibroin than of a heavier crêpe formerly described (13) shows that increasing the yardage per weight of

[1] Journal Paper No. J361 of the Iowa Agricultural Experiment Station, Ames, Iowa.

[2] The authors wish to thank Professor C. C. Major for calibration of the steam gauge.

TABLE 1. *Effect of steam on the weight, ash, and nitrogen of the fabrics*

| Temperature | Pressure of steam | Time | A. Iron-weighted silk | | | B. Lead-weighted silk | | | C. Tin-weighted silk | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Weight | Ash | Nitrogen | Weight | Ash | Nitrogen | Weight | Ash | Nitrogen |
| °C. | *pounds per sq. in.* | *hour* | *percentage of original fabric* | | | | | | | | |
| 100.0 | 0 | 1 | 99.3 | 43.6 | 8.17 | 99.0 | 43.8 | 9.05 | 99.1 | 51.5 | 9.22 |
| 112.6 | 8 | 1 | 99.2 | 43.6 | 8.15 | 98.9 | 43.7 | 9.05 | 98.8 | 51.3 | 9.09 |
| | | 2 | 98.0 | 43.1 | 8.09 | 96.0 | 42.7 | 8.88 | 96.3 | 50.1 | 8.38 |
| | | 3 | 95.6 | 41.2 | 7.67 | 93.8 | 41.7 | 8.04 | 93.9 | 48.4 | 8.12 |
| | | 4 | 93.5 | 40.0 | 7.53 | 89.9 | 40.8 | 6.57 | 92.1 | 47.2 | 7.74 |
| | | 5 | 93.3 | 39.9 | 7.31 | 88.9 | 40.3 | 5.63 | 89.2 | 46.0 | 7.47 |
| 124.1 | 18 | 1 | 98.0 | 41.3 | 8.09 | 95.9 | 41.1 | 8.86 | 95.6 | 50.1 | 8.16 |
| 132.9 | 28 | 1 | 95.4 | 40.3 | 7.64 | 88.8 | 40.1 | 5.64 | 89.9 | 47.3 | 7.55 |
| 140.2 | 38 | 1 | 92.8 | 38.3 | 7.28 | 86.8 | 38.2 | 5.08 | 88.0 | 46.2 | 5.99 |

TABLE 1. (Continued)

| Temperature | Pressure of steam | Time | D. Tin-lead-weighted silk | | | E. Zinc-weighted silk | | | F. Silk fibroin | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Weight | Ash | Nitrogen | Weight | Ash | Nitrogen | Weight | Nitrogen |
| °C. | *pounds per sq. in.* | *hour* | *percentage of original fabric* | | | | | | | |
| 100.0 | 0 | 1 | 99.2 | 51.6 | 8.45 | 98.9 | 50.1 | 8.08 | 99.7 | 18.61 |
| 112.6 | 8 | 1 | 99.0 | 51.0 | 8.42 | 98.8 | 50.1 | 8.05 | 99.4 | 18.37 |
| | | 2 | 96.9 | 49.4 | 8.34 | 98.2 | 50.1 | 7.98 | 99.3 | 18.29 |
| | | 3 | 93.2 | 47.7 | 7.68 | 97.0 | 50.2 | 7.03 | 99.1 | 18.23 |
| | | 4 | 92.8 | 47.2 | 7.54 | 90.1 | 49.0 | 6.08 | 98.4 | 18.19 |
| | | 5 | 91.6 | 46.9 | 7.39 | 85.9 | 47.9 | 5.32 | 98.0 | 18.16 |
| 124.1 | 18 | 1 | 96.8 | 49.3 | 8.29 | 95.9 | 50.0 | 7.80 | 98.9 | 18.25 |
| 132.9 | 28 | 1 | 93.6 | 47.7 | 7.54 | 88.0 | 48.4 | 5.91 | 97.4 | 18.15 |
| 140.2 | 38 | 1 | 90.8 | 45.3 | 7.15 | 84.7 | 46.7 | 4.96 | 96.4 | 17.77 |

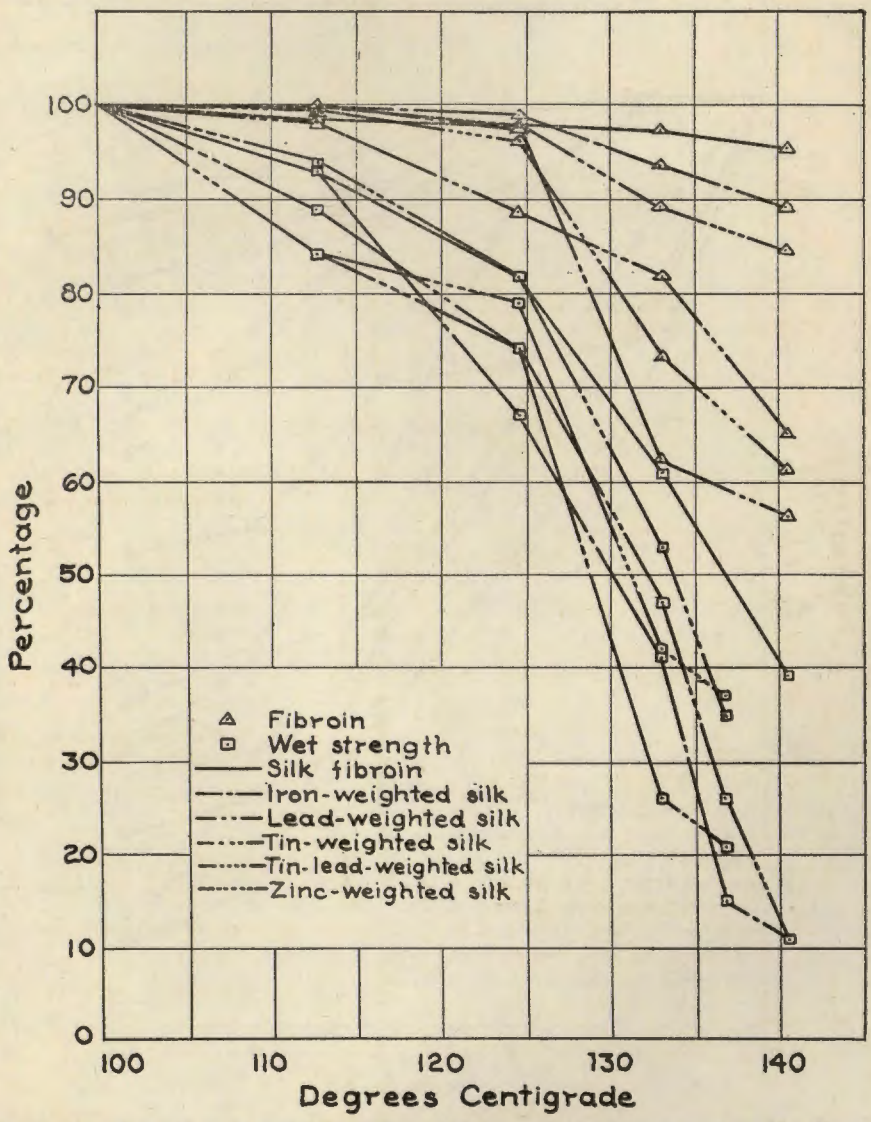


**Fig. 1.** Effect of steam at 0, 8, 18, 28, 33, and 38 pounds per square inch on the fibroin and wet warp breaking strength of the fabrics.

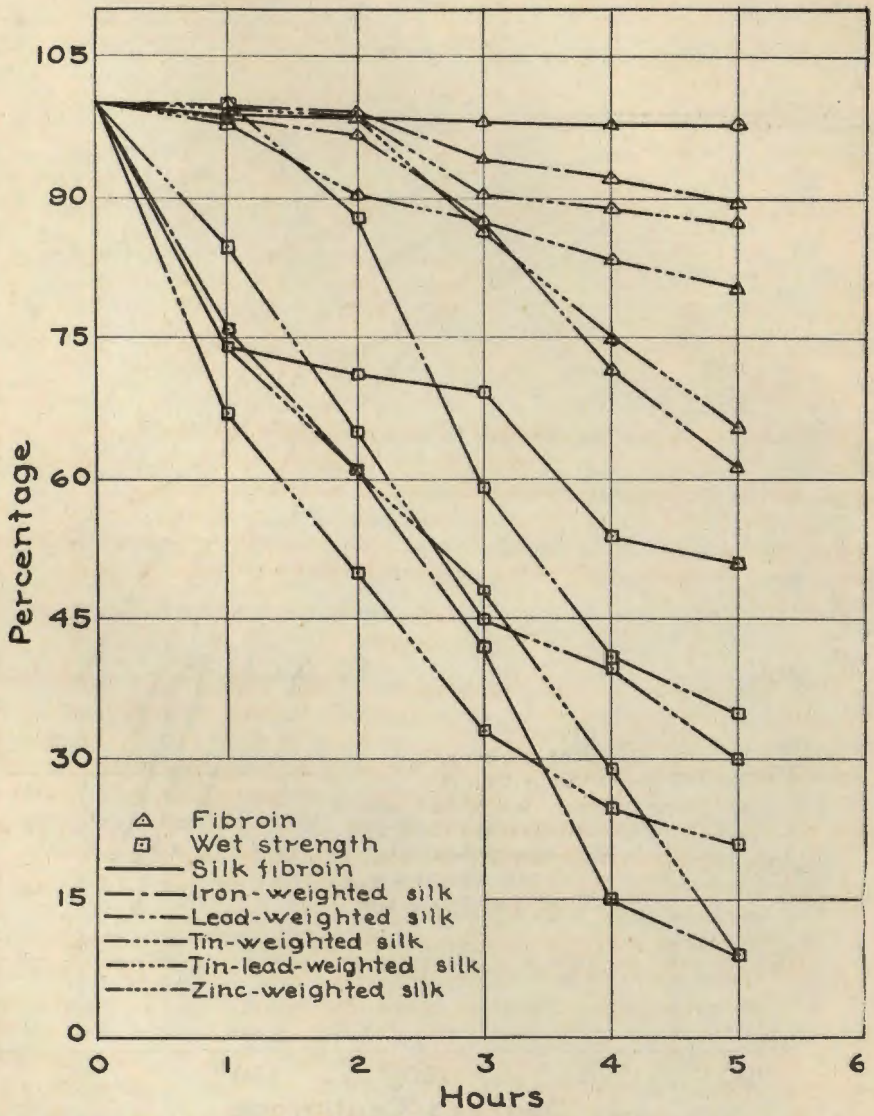


**Fig. 2. Effect of steam at 112.6° C. on the fibroin and wet warp breaking strength of the fabrics.**

TABLE 2. *Effect of steam on the wet warp breaking strength of the fabrics*

| Temperature | Pressure of steam | Time | Fabrics A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|
| °C. | *pounds per sq. in.* | *hour* | *pounds per inch* | | | | | |
| 100 | 0 | 1 | 27 | 19 | 19 | 17 | 19 | 28 |
| 112.6 | 8 | 1 | 25 | 17 | 16 | 16 | 17 | 26 |
| | | 2 | 20 | 15 | 13 | 12 | 14 | 25 |
| | | 3 | 14 | 10 | 9 | 8 | 11 | 24 |
| | | 4 | 5 | 7 | 8 | 6 | 5 | 19 |
| | | 5 | 3 | 6 | 6 | 5 | 2 | 18 |
| 124.1 | 18 | 1 | 18 | 14 | 14 | 14 | 15 | 23 |
| 132.9 | 28 | 1 | 11 | 5 | 9 | 9 | 8 | 17 |
| 136.7 | 33 | 1 | 4 | 4 | 5 | 6 | 7 | .... |
| 140.2 | 38 | 1 | 3 | <1 | 2 | <1 | <1 | 11 |

silk fibroin decreases its stability to steam. This raises the question as to whether the greater loss of strength by weighted silk may not be due in large part to its lower weight of silk fibroin per yard of fabric.

Tables 1 and 2 and figures 1 and 2 show that dilution of fibroin by weighting increases its conversion to soluble derivatives by steam and that loss in wet strength, with increasing temperature or time, is greater than this loss of fibroin.

## SUMMARY

1. The course of the degradation of silk fibroin and black iron-weighted, white lead-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silk fibroins by steam, in one hour at 0, 8, 18, 28, 33, and 38 pounds per square inch and in two, three, four, and five hours at eight pounds, has been followed by determination of the weight, ash, nitrogen, and wet strength of the residual plain-woven crêpes after thorough rinsing.

2. In one hour at 38 pounds the maximal decrease in percentage of ash, 6.3, occurred in the case of the tin-weighted silk; the zinc-weighted silk lost the most weight, 14.2 per cent.

3. The weighted silks, particularly the lead-weighted, became brown upon steaming one hour at eight pounds; the silk fibroin at 38 pounds.

4. The percentage conversion of the fibroin to soluble forms of nitrogen, in (a) one hour at 38 pounds and (b) five hours at eight pounds, has been shown of the order: lead-weighted (a, 43.9; b, 38.6), zinc-weighted (a, 38.6; b, 34.3), tin-weighted (a, 35.0; b, 19.3), tin-lead-weighted (a, 15.4; b, 12.6), iron- weighted (a, 10.9; b, 10.4), and silk fibroin (a, 4.5; b, 2.3). With increasing pressure, this conversion of the fibroin was greatest between 18 and 28 pounds, except that the tin-weighted silk lost most between 28 and 38 pounds; at eight pounds the greatest loss occurred between two and three hours, except in the case of the lead-weighted silk which lost most between four and five hours.

5. Although dilution of fibroin by weighting increases the conversion of its nitrogen to soluble forms by steam, the loss in wet strength,

with increasing temperature or time, exceeds the loss of fibroin. The tin-weighted silk alone withstood steam at a pressure of 18 pounds for one hour as well as silk fibroin; however, this weighted silk and the lead-weighted and zinc-weighted silks showed no measurable wet strength after one hour at 38 pounds. All the weighted silks withstood a pressure of eight pounds for one hour as well as silk fibroin, but upon continued steaming suffered decidedly greater losses.

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CONTENTS

Abstracts of Doctoral Theses



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NOTE: Complete copies of these theses may be consulted at the Library, Iowa State College, Ames, Iowa.

# THE FIREBRAT, THERMOBIA DOMESTICA (PACKARD), AND ITS GREGARINE PARASITES[1]

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Accepted for publication June 16, 1936

## PART ONE

## METHODS AND OBSERVATIONS IN REARING THE FIREBRAT, THERMOBIA DOMESTICA (PACKARD) (THYSANURA)

The apterygotous insects have been little used as experimental animals. The use of the common household lepismatids, the firebrat and the silverfish, is suggested. The firebrat is the larger and more tractable of the two, and it has the remarkable characteristic of being very thermophilic. Although fragile and slow of development, firebrats have proved excellent subjects for studies upon lepismatid biology, insect toxicology, thermophilia and thermoplegia, and gregarine parasites. They are suggested for use in studies upon regeneration, embryology, gametogenesis, insect nutrition, and animal sociology.

At Iowa State College firebrats are found associated with the heating system which provides nooks in the buildings and tunnels where temperatures above 30° C. prevail most of the year. From about one hundred captured specimens the author (1) has reared many generations, including thousands of descendants, over a four-year period. The insects are reared in glass culture dishes containing plaited strips of paper; in moving air at 37° C. and a relative humidity of about 70 per cent, and in semi-darkness. They are fed upon rolled oats, dried lean beef, dried brewer's yeast, cane sugar, and common salt, each material being supplied separately. They can also be reared, but more slowly, upon rolled oats alone or upon a basal diet of starch, casein, and "complete salt mixture," supplemented with dried yeast. Under favorable conditions firebrats reach maximum weight in about five months. Individuals of the same age show increasing variation in their sizes as they pass beyond the early instars. There is evidence that such structures as the metanotum grow as much as nine per cent in width during the third instar. The sexes cannot be distinguished, without minute examination, until the eighth instar. The males and females are about equal in number. With regard to the sexual habits the writer agrees with Spencer (3) that copulation is absent and that the females take up spermatophores dropped by the males. Balls of sperm have been found in the genital tracts of females. There is no oviposition in colonies from which males are absent and oviposition ceases in a few days following removal of males. When hungry, firebrats are fairly tolerant of moderate light. When protected by glass from air-currents (which excite them strongly) they feed and oviposit in the normal daylight of the laboratory table. There is evidence that these insects

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[1] Original thesis submitted December, 1935. Doctoral thesis number 355.

are somewhat gregarious, although they usually avoid direct contacts with each other. Fighting and cannibalism occur under stress of hunger.

## PART TWO

## THE TEMPERATURE RELATIONS OF THE FIREBRAT, THERMOBIA DOMESTICA (PACKARD) (THYSANURA)

The ,rebrat, *Thermobia domestica* (Pack.), has been recognized as a heat-loving insect ever since it became known to science over 60 years ago. It is restricted to the vicinities of hearths, ovens, heat conduits, and to other habitats where temperatures above 30° C. prevail. In contrast to the temperature relations of the firebrat those of the larvae of the corn borer, *Pyrausta nubilalis* Hubn., are cited from unpublished studies. The borer thrives at ordinary summer temperatures near 27° C. The larvae were killed in laboratory experiments by one-hour exposure in air at a temperature of 48° C. and survived prolonged exposure to temperatures in the winter of southern Ontario. Experiments upon the firebrat were aimed at determining its maximum and minimum fatal temperatures, the range of its preferred temperatures, and its thermotactic optimum. The latter is defined as the temperature the insect is most likly to choose by thermotactic responses when offered access to a range of suitable environments differing only in temperature. A thermogradient was constructed, the principal part of which was a metal trough divided into transverse compartments separated by narrow openings. Each compartment was covered and so equipped that the insects might live in it indefinitely if suited with the temperature. When heat was applied at one end a rough, variable gradient of temperatures was obtained in the succeeding compartments. Firebrats in such a device showed that they strongly avoided temperatures outside the range of 32 to 43° C. The mean point of the distribution, which is regarded as the thermotactic optimum, was 37.5° C. In other experiments it was found that firebrats would not breed at 24.5° C. and only very slowly at 29.5° C. Oviposition occurred at 42° C. but not at 45° C. Eggs at 37° C. hatched in thirteen or more days, and at 42° C. in nine or more days. The life-cycle from egg to egg was at least four weeks shorter at 42° C. than at 37° C. In a variety of preliminary tests nymphs of the second instar and fully grown firebrats were removed from their favorable environment in the incubator and subjected to extreme temperatures. It was found that exposure to — 7° C. for one hour, or to 2° C. for less than twenty-four hours, was sufficient to kill nearly all the animals so tested. Firebrats can live for many days at 45° C. without apparent paralysis. At 47° C. adults retain the power of locomotion for at least ten hours. The nymphs are slightly less resistant to high temperatures. An exposure to 49° C. for one hour causes thermoplegia and is usually fatal, both to nymphal and to mature firebrats.

## PART THREE

## THE GREGARINE PARASITES OF THE FIREBRAT, THERMOBIA DOMESTICA (PACKARD) (THYSANURA)

Two species of septate gregarines became numerous in cultures of the firebrat. As parasites of the firebrat these gregarines are convenient for continuous cultivation in the laboratory. A description, more detailed

than that previously published (2), is given for each species. Firebrats in cultures containing one or both species of gregarines seem to thrive as well as others reared gregarine-free from the egg.

### *Lespismatophila thermobiae* Adams and Travis

As many as two hundred and fifty trophozoites have been taken from the caeca of one firebrat. The mature sporonts move toward the posterior end of the ventriculus to encyst in pairs. When the cysts are deposited by the host the gametocytes are usually hemispheroidal with a discoidal, hyaline layer between them. The latter disappears in the first day of the exogenous cycle. At 34.5° C. the cyst acquires a grayish tint on the third day, owing to the coloration of the developing sporocysts. On the fourth day the cyst becomes grayish black in color. On the fifth or sixth day it forcibly bursts, everting a ball of closely coiled spore-chains. The latter rapidly uncoil and extend until they appear as a dark, fluffy mass lying over the remnant of the ruptured cyst. At 43° C. dehiscence comes in three days. Spores retain their infective power for at least three months.

### *Colepismatophila watsonae* Adams and Travis

The trophozoites are crowded between the peritrophic membrane and the ventricular epithelium. Sometimes they nearly block the lumen and sometimes they distend the ventricular wall. The larger sporonts frequently occupy deep cavities in the epithelium; some of these cavities have been found to extend to the basement membrane, indicating the displacement or destruction of many cells. The shortest life-cycle period determined at 33° C. is eight days. The average number of spores in a cyst is estimated, from partial counts, to be about sixteen hundred.

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1935. J. Parasitol., 21:56-59.

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1930. Canad. Entomol., 62:1-2.

# THE HEAT CAPACITY OF IRON CARBIDE[1]

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Accepted for publication June 16, 1936

The free energy change accompanying the formation of a compound from its elements is a criterion of the stability of the compound. The increase in free energy, $\Delta F$, may be found by the equation,

$$\Delta F = \Delta H - T\Delta S,$$

where H is heat content, T is absolute temperature, and S is entropy.

$\Delta H$ for the formation of $Fe_3C$ has been determined a number of times. The results of Brodie, Jennings and Hayes, as recalculated by Yap and Liu (1), will be used in the following calculations. Values for the entropy of iron (2) and of carbon (3) have already been calculated from heat capacity data. The only additional information necessary for the calculation of $\Delta F$ is the heat capacity of $Fe_3C$ from absolute zero up to the temperature at which $\Delta F$ is desired.

Naeser (4) has recently made some measurements on the heat capacity of iron carbide at low temperatures, but made no calculation of entropy. He obtained his data using pure $Fe_3C$ and a water calorimeter, while in the present investigation the measurements were made on $Fe_3C$ as it exists in steel, using the method of electrical heating in a vacuum.

## MATERIAL APPARATUS, AND METHODS OF PROCEDURE

The steel samples were prepared by melting Armco iron and pure carbon in magnesia crucibles. The slugs thus produced were examined microscopically. No inclusions were observed and the carbide seemed to be present as pearlite and massive cementite. The slugs were turned down to .75 inch in diameter and about 2.31 inches in length, leaving at one end a small additional piece of metal, through which a hole was bored for suspending the sample. An Armco iron sample of the same dimensions, which was used for standardizing the calorimeter, was made from .75 inch Armco iron rod. Each slug was wound with 40 ohms of constantan wire. A coat of Bakelite lacquer was applied to give thermal contact.

The calorimeter was made entirely of Pyrex glass. It was evacuated by a mercury vapor pump in series with a mechanical pump. The sample was suspended by a silk thread from the copper wires which served as leads to the heating coil. The energy input was measured by the known resistance and the current was measured by a milliammeter.

In most cases temperature was measured by a previously calibrated thermocouple. The calorimeter was surrounded by a constant temperature bath (ice, liquid oxygen, or solid $CO_2$) and the rates of heating and of cooling were measured by the thermocouple.

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[1] Original thesis submitted July, 1935. Doctoral thesis number 332.

An adiabatic method was used for the runs at room temperature and above. A water bath surrounding the calorimeter was kept at the same temperature as the sample by using a differential thermocouple. The temperature was measured by a thermometer.

## CALCULATIONS AND RESULTS

In the adiabatic runs, all the electrical energy was used in heating the sample. The heat capacity of a slug, $C_p$, was calculated from the rate of rise of temperature, dT/dt, and the energy imput in calories per minute.

$$C_p = \frac{(I^2R)\,(60)}{4.1826}\,\frac{dt}{dT}$$

dt/dT was found by measuring the slope of the curve obtained by plotting time in minutes against temperature.

In the method in which the calorimeter was surrounded by a constant temperature bath, part of the electrical energy was used in heating the sample and part was lost to the surroundings. The heat capacity was calculated by the equation,

$$C_p = \frac{(I^2R)\,(60)}{(4.1826)\left(\frac{dE}{dt}+\frac{dE'}{dt}\right)\left(\frac{dT}{dE}\right)}$$

dE/dt is the increase per minute of the e. m. f. of the thermocouple, dE'/dt is the similar rate of decrease on cooling, and dE/dT is the rate of change of temperature with respect to e. m. f. of the thermocouple as given by the calibration curve for the thermocouple.

The specific heat was calculated for each slug. The specific heat of $Fe_3C$ was calculated by plotting specific heat against percentage of carbon and extrapolating to pure $Fe_3C$ (6.6 per cent C). The following table gives specific heat and molal heat capacity at several temperatures. These are somewhat lower than the corresponding values obtained by Naeser.

| Absolute temperature | Specific heat | Molal heat capacity |
|---|---|---|
| 323.2 | 0.1358 | 24.38 |
| 303.2 | 0.1341 | 24.06 |
| 280.7 | 0.1301 | 23.34 |
| 225.8 | 0.1160 | 20.83 |
| 201.3 | 0.1087 | 19.51 |
| 161.2 | 0.0884 | 15.87 |
| 120.5 | 0.0694 | 12.46 |
| 102.4 | 0.0566 | 10.16 |

The entropy of iron carbide was obtained by plotting absolute temperature against molal heat capacity divided by temperature and integrating graphically by finding the area under the curve. The heat capacity values used for temperatures below 100° K. were obtained by comparing

the temperature-specific heat curve for $Fe_3C$ to that for iron. Since the two are quite similar in the range investigated experimentally and approach more closely at low temperatures, it was assumed that near absolute zero the form of the curve for $Fe_3C$ would be very much like that for iron. The entropy of $Fe_3C$ at 25° C. was found to be 23.55 units.

For the formation of one mole of $Fe_3C$ from its elements at 25° C.,

$$\Delta S = 23.55 - (3 \times 6.60 + 1.39) = 2.36 \text{ units.}$$

$$\Delta F = 12300 - (298.16)(2.36) = 11596 \text{ cal.}$$

This indicates that $Fe_3C$ is unstable or metastable at room temperature.

## SUMMARY

1. The heat capacity of iron carbide in steel has been determined from 102° K. to 323° K. (see table).

2. The entropy of $Fe_3C$ at 25° C. is found to be 23.55 units.

3. The free energy increase accompanying the formation of one mole of $Fe_3C$ from its elements at 25° C. is calculated to be 11596 cal.

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# EFFICIENCIES OF PETROLEUM DISTILLATES AS COOLING MEDIA FOR INTERNAL COMBUSTION ENGINES[1]

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Since various individuals have used petroleum distillates as cooling media for their automobile engines at various times and under various conditions, without knowing the effects of such cooling, it was considered logical, since the subject appears not to be recorded in the literature, to investigate the effects of petroleum distillate, such as kerosene or distillate, upon the motor and the cooling system.

Six different kerosenes were obtained on the market and were compared with radiator alcohol, radiator glycerine and radiator glycol. The distillation range of these materials is presented in table 1.

At 0° C., the viscosity of kerosene (38 seconds) is only slightly greater than that of water (34 seconds) but is less than that of a 50-50 mixture of radiator alcohol (51 seconds), radiator glycol (56 seconds) or radiator glycerine (58 seconds). At — 32° C., the kerosene has definitetly lower viscosity (58 seconds) than the mixtures of glycol (168 seconds), alcohol (363 seconds) or glycerine (which froze at — 27° C.).

The flash and fire points of the various kerosenes (150° F. and 170° F., respectively, in the open cup) were definitely higher than those of a 50-50 alcohol mixture (66° F. and 78° F., respectively).

Corrosion tests were run on both tinned copper and aluminum strips with the result that the kerosenes are no more corrosive than good tap water, and far less corrosive than radiator alcohol.

Rubber radiator hose, both a poor (black) and a good (red) grade, was subjected to the action of the various solutions at high and low temperatures. These results are presented in tables 2 and 3. The significant difference appeared in the bursting strength. The black hose was badly decayed and broke at 25 per cent normal bursting pressure, whereas the red hose remained firm and broke at 65 per cent normal bursting pressure.

Motor tests were made to determine the rate of heating of the motor when using water, alcohol, kerosene and glycol. The kerosene caused the motor to heat quicker, but when equilibrium was reached, the kerosene caused only a slight increase in operating temperature. Road tests also showed the same results.

The following conclusions are drawn from the data:

1. Kerosene is less viscous at low temperatures than any of the materials commonly used as cooling media.

2. Kerosene is less dangerous as a fire hazard than a 50 per cent alcohol solution.

3. Kerosene offers no corrosion problem when in contact with metal surfaces.

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TABLE 1. *Distillation range (°F.) of anti-freeze solutions*

| | Kerosenes | | | | | | Radiator | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | Alcohol | Glycerine | Glycol |
| Initial | 330 | 352 | 348 | 346 | 350 | 369 | 168 | 219 | 323 |
| 10% | 392 | 410 | 382 | 391 | 382 | 401 | 170 | 219 | 368 |
| 20% | 404 | 422 | 393 | 404 | 392 | 414 | 170 | 219 | 374 |
| 30% | 414 | 432 | 404 | 414 | 400 | 420 | 170 | 219 | 374 |
| 40% | 422 | 439 | 412 | 422 | 408 | 426 | 170 | 219 | 374 |
| 50% | 429 | 446 | 420 | 432 | 416 | 432 | 170 | 288 | 374 |
| 60% | 436 | 453 | 430 | 441 | 424 | 438 | 170 | 492 | 375 |
| 70% | 443 | 461 | 441 | 450 | 434 | 444 | 171 | 522 | 376 |
| 80% | 453 | 470 | 454 | 460 | 444 | 454 | 171 | 538 | 376 |
| 90% | 468 | 485 | 470 | 476 | 469 | 468 | 172 | 620 | 378 |
| End | 485 | 514 | 494 | 485 | 504 | 499 | 368 | Decomp. | 410 |
| Recovery | 98.0 | 98.5 | 98.6 | 98.9 | 98.0 | 98.0 | 98.0 | 98.0 | 96.5 |
| Residue | 1.5 | 1.5 | 1.4 | 0.2 | 1.6 | 1.2 | 0.9 | ..... | 1.3 |
| Loss | 0.5 | 0.0 | 0.0 | 0.0 | 0.4 | 0.8 | 1.1 | ..... | 2.2 |

TABLE 2. *Effect of various solutions on black radiator hose (48 days)*

| Hose lbs. | Solution | Temp. °F. | Bursting pressure lbs./in.² | Increase in Wt. Pctg. | Increase in Vol. Pctg. | Nature of failure |
|---|---|---|---|---|---|---|
| 15 | Kerosene | 170 | 30 | 22.5 | 50.6 | Inside badly decayed, leak occurred, through fabric; could not burst it. |
| 9 | " | 170 | 60 | 23.6 | 47.1 | Inside partially decayed; break normal short spiral split. |
| 13 | " | 115 | 55 | 19.6 | 37.9 | |
| 2 | " | 115 | 75 | 24.0 | 46.5 | |
| 17 | Glycol | 170 | 105 | 3.6 | 3.6 | |
| 10 | " | 170 | 95 | 3.7 | 5.3 | |
| 5 | " | 115 | 90 | 1.5 | 5.7 | |
| 4 | " | 115 | 105 | 4.2 | 6.8 | |
| 11 | Water | 170 | 140 | 5.1 | 7.2 | Rubber firm, break normal short spiral split of outer fabric followed by tearing of rubber. |
| 19 | " | 170 | 90 | 5.2 | 7.1 | |
| 8 | " | 115 | 110 | 1.9 | 3.3 | |
| 20 | " | 115 | 135 | 1.8 | 2.1 | |
| 6 | Glycerine | 170 | 140 | 1.2 | 3.6 | |
| 16 | " | 170 | 125 | 1.3 | 2.1 | |
| 3 | " | 115 | 125 | 0.3 | 2.1 | |
| 12 | " | 115 | 145 | 1.0 | 2.8 | |
| 7 | Alcohol | 170 | 110 | 0.8 | 5.7 | |
| 18 | " | 170 | 110 | 4.3 | 6.5 | |
| 1 | " | 115 | 145 | 3.3 | 4.2 | |
| 14 | " | 115 | 135 | 2.8 | 3.6 | |
| Original | ..... | ..... | 125 | ..... | ..... | |

4. Kerosene is more severe than other cooling media on rubber hose, but a quality product stands up both in laboratory tests and under actual conditions.

5. Kerosene causes a motor to heat up slightly faster than the other solutions and attains a temperature of 15° F. higher under no load.

6. Kerosene can be used satisfactorily as a cooling medium in actual operation, even in quite warm weather, causing the motor to run about 30° F. hotter than with water.

TABLE 3. *Effect of various solutions on red radiator hose (48 days)*

| Hose lbs. | Solution | Temp. °F. | Bursting pressure lbs./in.² | Increase in Wt. Pctg. | Increase in Vol. Pctg. | Nature of failure |
|---|---|---|---|---|---|---|
| 17 | Kerosene | 170 | 105 | 33.1 | 54.6 | In all cases the rupture was a short spiral split of the outer fabric, followed by tearing of the rubber. |
| 19 | " | 170 | 150 | 29.9 | 49.2 | |
| 18 | " | 120 | 240 | 6.3 | 7.5 | |
| 20 | " | 120 | 230 | 4.3 | 7.1 | |
| 16 | Glycol | 170 | 195 | 4.4 | 3.5 | |
| 10 | " | 170 | 240 | 3.6 | 4.7 | |
| 13 | " | 120 | 250 | 0.6 | 0.0 | |
| 8 | " | 120 | 250 | 3.5 | 1.3 | |
| 11 | Water | 170 | 250 | 7.3 | 8.4 | |
| 2 | " | 170 | 245 | 8.9 | 10.0 | |
| 5 | " | 120 | 250 | 1.6 | 0.0 | |
| 15 | " | 120 | 230 | 1.7 | 0.0 | |
| 4 | Glycerine | 170 | 300 | 4.5 | 3.9 | |
| 12 | " | 170 | 255 | 1.7 | 0.0 | |
| 9 | " | 120 | 260 | 0.3 | 0.0 | |
| 6 | " | 120 | 300 | 0.9 | 0.0 | |
| 3 | Alcohol | 170 | 270 | 2.4 | 0.7 | |
| 1 | " | 170 | 250 | 1.5 | 0.7 | |
| 7 | " | 120 | 250 | 15.8 | 14.8 | End swelling due to outside leak. |
| 14 | " | 120 | 260 | 3.3 | 0.7 | |
| Original | ..... | ..... | 200 | ..... | ..... | |

# THE EFFECT OF HIGH FREQUENCY EXCITATION UPON THE INTENSITIES OF SPECTRAL LINES[1]

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High frequency electric discharges in hydrogen were excited by voltages applied to a discharge tube by means of external electrodes. The spectrum of the discharge was studied as a function of the applied frequency. In particular the relative intensities of the Balmer series were measured for a series of frequencies and pressures.

The source of high frequency potential was that developed across the plate tank circuit of a push-pull oscillator which used No. 852-type tubes. The radio-frequency voltage was applied to the discharge tube by means of sleeve electrodes of fine wire. The applied potential difference was calculated from known values of current, capacity, and frequency of oscillation in the tank circuit. A constant peak potential of 1600 volts was maintained at the electrodes which were spaced 30 cm. apart. Excitation wavelengths of from 5.1 to 32.5 meters were used.

The gas system was designed for maximum purity. The hydrogen, generated electrolytically, was dried in a $P_2O_5$ trap and admitted to a chamber containing a palladium diffusion tube. Passage of the gas into the discharge tube was controlled by varying the temperature of the palladium tube, this being accomplished by passing an electric current through it. Diffusion pumps using Apiezon oil "B" were used and a Pirani gauge previously calibrated for hydrogen was used for pressure measurements. The whole vacuum system was of Pyrex glass except for a Corex window sealed in the end of the discharge tube. All joints up to the stop-cock at the pumps were glass sealed. Impurities due to wax and grease were thus eliminated and thorough baking of the entire tube at 500°. C. was possible. A trap between the discharge tube and pumps was cooled with solid $CO_2$ and acetone.

The discharge tube was 4.8 cm. in diameter and 75 cm. long. This length was greater than the theoretical minimum length for the frequencies and pressures used as calculated from J. Thomson's[2] equation for the initiation potential for this type of gaseous discharge.

$$E > \phi(f)\left[\frac{Vp}{K} + \frac{4\pi^2 f^2 K}{2p\, e/m}\right]$$

where E represents the electric field, V the ionization potential of the gas, $\frac{K}{p}$ — the mean free path of an electron, f the frequency of oscillation, and

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[1] Original thesis submitted July, 1935. Doctoral thesis number 331.

[2] Thomson, J. 1930. On the mechanism of the electrodeless discharge. Phil. Mag. (7). 10:280-291.

e/m the ratio of charge to mass for the electron. $\phi(f)$ is a function which represents loss of ions due to diffusion and which probably varies inversely with the square of the frequency of excitation. This function would also vary with different geometrical conditions such as tube dimensions.

The range of pressures studied in this investigation was from 300 to 4 microns. Beyond these limits it was impossible to obtain data for all the desired excitation wavelengths and maintain a constant potential at the electrodes.

Intensity ratios were obtained from spectograms taken in conjunction with a logarithmic sector disc which gives spectral lines whose differences in length are proportional to their intensity ratios. Line lengths were measured to 0.1 mm. consistently, this corresponding to an accuracy of not more than 10 per cent error in the value of an intensity ratio. Spectograms were taken with a Bausch and Lomb medium quartz spectograph. W. and W. panchromatic plates of the same emulsion batch were used and were developed with developer formula D-19 from the same stock solution under the same conditions of temperature and time.

Considerable difficulty was encountered in attempting to rid the discharge of all spectoroscopic traces of impurities. The most persistent of these appeared in the ultra-violet region and were the 3085Å water-vapor band and the 2882Å and 2899Å $CO_2$ bands, the latter appearing only at pressures of a few microns or less. Baking of the glass tube had no effect upon the appearance of these impurities. The water-vapor band could be eliminated by baking and pumping only if no hydrogen were present. It is believed that it results from the combination of hydrogen with oxygen released from the $SiO_2$ of the glass walls by electron bombardment. The $CO_2$ appearing at low pressures perhaps also results from some similar action.

A series of spectograms of the discharge was obtained for a range of frequencies between 5.1 and 32.5 meters, while the pressure and applied potential difference were held constant. Data for six pressures of from 5 to 300 microns were obtained.

The results of the investigation show that:

1. There is a distinct variation in intensity ratios with excitation frequency.

2. There is a region of excitation frequencies which gives a minimum excitation of eac hterm with respect to $H\alpha$ and that this region of frequencies depends upon pressure and the term under consideration.

3. Ionization efficiency and intensities of the higher terms of the series vary (more or less directly) with each other if cumulative ionization is negligible.

4. Intensities of the higher terms of the Balmer series increase as the pressure decreases to about 10 microns. For further diminishing of pressure $H\beta$ and $H\gamma$ continue to increase in intensity while the higher terms decrease.

5. Population of the energy states giving $H\beta$ and $H\gamma$ increase with decreasing pressure but vary relatively with the frequency of excitation voltage.

6. A value, $e^{-.1}\left(\frac{f}{10^7}\right)^2$, can be chosen empirically for $\phi(f)$ of J.

Thomson's equation for the initiating potentials for this type of discharge. With this function the equation yields curves which are in good general agreement with the experimental results.

7. Some gas impurities appearing in the ultra-violet and not apparent in the visible region are inherent in a hydrogen discharge excited by high frequency potential and are probably due to electronic bombardment of the glass.

# THE DIELECTRIC CONSTANT AND THE SPECIFIC CONDUCTANCE OF PURE LIQUID HYDROGEN SULPHIDE[1]

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## I. THE PRODUCTION OF PURE HYDROGEN SULPHIDE

The hydrogen sulphide gas was produced by the method of Habermann (1). This method utilizes the reaction between solid calcium sulphide and a saturated solution of magnesium chloride Randall and Bichowsky (2) report that "the gas (hydrogen sulphide) is probably purer than that prepared by any other known method." The hydrogen sulphide gas was washed with water, barium sulphohydrate suspension, dried with calcium chloride, aluminum sulphide, and phosphorus pentoxide. It was then passed through a baffle for the removal of entrained solids, liquefied at atmospheric pressure by means of a dry ice-ether mixture, and finally fractionally distilled into the test cell.

## II. THE A. C. BRIDGE METHOD

The conventional Kohlrausch method for determining electrical conductance employs a parallel arrangement of capacitance and resistance for balancing the impedance of the filled test cell. Difficulties are introduced into this method when the balancing resistance becomes quite large (500,000 ohms and larger) because of the impossibility of producing wire wound resistors of this magnitude that are reactance free. Since the specific resistivity of liquid hydrogen sulphide was known to be quite large (3) (4) (5) (6) it was found expedient to measure the power ratio, $N_x$ (ratio of equivalent series resistance to reactance) of the liquid by means of Siskind's capacitance bridge (7) and to compute the equivalent parallel resistance, $R_p$, by the aid of the following equations:

$$N_x = R_x\overline{C_x} \tag{1}$$

$$R_x = N_x/\overline{C_x} \tag{2}$$

$$R_p = 1/\omega^2\overline{C^{+2}}_xR_x \tag{3}$$

In the above equations

$N_x$ = power ratio

$R_x$ = equivalent series resistance

$\overline{C_x}$ = capacitance of filled test cell

$\omega = 2\pi$ x frequency in c.p.s.

In regard to equation 3 see Hague (8), page 136.

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## III. THE CELL CONSTANT

The following method was used in determining the cell constant of the test cell. It is proposed to avoid difficulties encountered in the evaluation of the cell constant for a cell of large plate area and of small plate separation.

The cell constant, $k_c$, of a cell whose plate separation is d and whose uniform cross section is A, is expressed by the following equation:

$$k_c = \frac{d}{A} \tag{4}$$

The capacitance in micro-micro farads of a parallel plate condenser (when air filled) whose plate separation is d, and whose uniform cross section is A is expressed by

$$C_{\mu\mu_F} = \frac{1}{(.9)4} \times \frac{A}{d\pi} \tag{5}$$

Solving equation 5 for d/A it is found that the cell constant, $k_c$, is

$$k_c = \frac{d}{A} = \frac{0.08841}{C_{\mu\mu_F}} \tag{6}$$

This derivation was extended to the case of a cell having coaxial cylinders for electrodes with the result that

$$k^0_c = \frac{0.08841}{C_{\mu\mu_F}} \tag{7}$$

This equation (7) holds rigorously when the distance between the electrodes is small compared to the diameter of the cylinder.

## IV. THE SCREENED AUDIO-FREQUENCY CAPACITANCE BRIDGE

Siskind's (7) network was employed. The component parts were contained in individual compartments of an earthed copper alloy box. The oscillator and detector were contained in individual earthed copper alloy shields, and were connected to the bridge proper by means of duplex cables in earthed shields.

1. *The oscillator.* A vacuum tube generator similar in design to that of Shedlovsky (9) was used.

2. *The detector.* Western Electric headphones and a two-tube resistance coupled amplifier were employed in the detecting device.

3. *The Wagner-Ground circuit.* This unit was composed of two Ayrton-Perry coils, a ten ohm rheostat, and two 250$\mu\mu_F$ condensers.

4. *The ratio arms.* This unit was a standard part from the General Radio type 216 capacity bridge (10).

5. *Balancing condensers.* These condensers were General Radio products with the exception of one Leeds and Northrup standard con-

denser. The General Radio Precision Condenser used for the determination of capacitances of the test specimens was calibrated so that a capacitance *difference* of $0.2\mu\mu_F$ or 0.1 per cent (whichever was the larger) could be detected.

## V. THE TEST CELL

The test cell was essentially the same as that described by Field (11). It was constructed of nickel electrodes provided with guard rings. The important feature in the design of this cell is the absence of solid supports between the high and low potential electrodes. Such a cell as this exerts no shunting effect upon the liquid being measured. The "direct capacitance" of this cell was $40.4_0\mu\mu_F$. After substituting this value in Equation 7, the cell constant was found to be $2.18_8 \times 10^{-8}$.

## VI. THE ELECTRICAL CONSTANTS FOR PURE LIQUID HYDROGEN SULPHIDE

The dielectric constant of a liquid is found by the radio of the "filled" to the "empty" capacitance of the test cell.

The following values were found for the constants of hydrogen sulphide at a temperature of — 78.5° C.

Dilectric constant—$9.0_5$

Specific conductance—$3.1 \times 10^{-10}$ohm$^{-1}$cm.$^3$

The following values have been reported previously.

Specific conductance

| | | |
|---|---|---|
| | $0.1 \times 10^{-6}$ ohm$^{-1}$ cm.$^3$ | (3) |
| less than | $4.0 \times 10^{-7}$ " " | (4) |
| | $3.7 \times 10^{-11}$ " " | (5) |
| | $1.0 \times 10^{-11}$ " " | (5) |

Dilectric constant

| | |
|---|---|
| 10.2 | (4) |
| 9.4 | (12) |

Probably the best previous measurements on the specific conductance were made by Wilkinson and co-workers (5) (6) in this laboratory. Their reported values are somewhat less than the present observation. This is to be expected in view of the fact that they employed a direct current galvanometer method and encountered some polarization. Taking this into account the agreement between the new and old data from this laboratory is good.

Kemp and Dennison (12) observed the dielectric constant at the melting point (— 82.5° C.) while the present observation is made at — 78.5° C., which perhaps accounts for the difference, although Kemp and Dennison claim only a precision of ten per cent. Obviously the value of Magri (4) is widely in error.

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# PHYSIOLOGICAL STUDIES AND CLASSIFICATION OF THE BUTYRIC ACID-BUTYL ALCOHOL BACTERIA[1]

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Morphological, cultural and physiological characteristics of 36 strains of butyric acid-butyl alcohol bacteria were investigated. The following seven species were differentiated. *Clostridium amylobacter, Cl. acetobutylicum, Cl. felsineum, Cl. butyricum, Cl. saccharobutyricum, Cl. Pasteurianum* and *Cl. Beijerinckii.*

All the species are anaerobic, sporulating, rod-shaped, gram positive and catalase negative. Granulose is present during the clostridial stage. Nitrates are not reduced and indol is not formed. $H_2S$ is produced from thiosulphates. The characteristic products of dissumulation are butyric and acetic acids, butyl alcohol, $CO_2$ and $H_2$. Glucose, sucrose, lactose, maltose, xylose, levulose, mannose, galactose, amygdalin, salicin and cellobiose are fermented. Adonitol, dulcitol and erythritol are not attacked.

Acetylmethylcarbinol is a normal product of dissimilation by *Cl. acetobutylicum* and *Cl. felsineum* but 2,3-butyleneglycol is not produced. In the presence of peptone and glucose the carbinol is not reduced.

Acetomethylcarbinol does not occur as an end product of dissimilation by *Cl. butyricum, Cl. amylobacter, Cl. saccharobutyricum, Cl. Pasteurianum* and *Cl. Beijerinckii.* 2,3-Butyleneglycol is normally produced. Added carbinol is completely reduced to the glycol.

The fermentation of 5 per cent corn mash by *Cl. felsineum* and *Cl. acetobutylicum* was characterized by extensive hydrolysis of proteins and starch, with the formation of high yields of butyl alcohol and acetone. Of the weight of corn fermented, 20 to 30 per cent was converted to neutral volatile products. The remaining species were relatively non-proteolytic. Corn mash was only partially fermented with the formation of mostly acid products.

Tatum, Peterson and Fred (1934, 1935) reported that asparagin stimulated the production of butyl alcohol from the fermentation of corn mash by butyric acid anaerobes. No increase in other neutral products was observed.

In the investigation reported here, it was found that only *Cl. butyricum* was stimulated by the addition of asparagin to corn mash. Moreover, in the presence of asparagin, peptone, yeast extract and mixtures of amino acids, corn mash was converted into as much as 17 per cent butyl and 9 per cent isopropyl alcohols. The addition of these nitrogenous substances to corn mash was without effect on the yields of "solvents" by the other species. Although *Cl. butyricum* was unable to utilize undegraded proteins as source of nitrogen, no specific amino acids appeared to be essential to the metabolism of this species.

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Pyruvic acid, which has been suggested by several investigators as an intermediate product in the butyric acid-butyl alcohol fermentation, was converted by cell suspensions of *Cl. butyricum* into acetic and butyric acids, $CO_2$ and $H_2$. Hydrogen, donated by pyruvic acid, was available for the reduction of butyric acid and acetone to butyl and isopropyl alcohols respectively. Lactic and formic acids were not attacked by cell suspensions of this species.

The following key for the differentiation of the species was proposed:

A. Bacteria producing considerable amounts of butyl alcohol, acetone and ethyl alcohol from corn without added nitrogenous substances. Strongly proteolytic. Gelatin liquefied. Pink or orange-yellow pigment produced in corn mash. Melibiose and trehalose not attacked. 2,3-Butyleneglycol not formed. Voges-Proskauer positive.

B. Not fermenting raffinose, rhamnose, inulin and pectin. Fermenting mannitol and α-methylglucoside. Orange-yellow pigment in corn mash.

*Clostridium acetobutylicum.*

BB. Fermenting raffinose, rhamnose, inulin and pectin. Not fermenting mannitol and α-methylglucoside. Pink pigment in corn mash.

*Clostridium felsineum.*

AA. Producing considerable amounts of butyl and isopropyl alcohols from corn when an available source of nitrogen is present. Non-proteolytic. Gelatin not liquefied. Voges-Proskauer negative. 2,3-Butyleneglycol produced. No pigment produced. Melibiose, trehalose and rhamnose fermented.

*Clostridium butyricum.*

AAA. Very small amounts of alcohols produced from corn with or without added nitrogenous substances. Non-protelytic. Gelatin not liquefied. Rhamnose not fermented.

B. Fermenting starch, dextrin, glycogen and glycerol.

C. Fermenting melezitose, sorbitol, inulin, arabinose and mannitol. Not fermenting pectin.

*Clostridium Pasteurianum.*

CC. Not fermenting melezitose, sorbitol, inulin, arabinose and mannitol.

D. Not fermenting pectin.

*Clostridium saccharobutyricum.*

DD. Fermenting pectin.

*Clostridium amylobacter.*

BB. Not fermenting starch, dextrin, glycogen and glycerol.

*Clostridium Beijerinckii.*

In view of the probability that *Bacillus amylobacter* Van Tieghem (1877) was a pectin fermenting organism and since this specific name

should be recognized for the type species of the genus *Clostridium* Prazmowski (1880), it was proposed that the "retting organism" previously designated as *Plectridium pectinovorum* Stormer (1903), *Granulobacter pectinovorum* Beijerinck and Van Delden (1904) and *Cl. pectinovorum* Donker (1926), be named *Clostridium amylobacter* and be recognized as the type species. Moreover, it was proposed that the isopropyl alcohol producing species be designated at *Cl. butyricum* Prazmowski since it is highly probable that this specific name was originally assigned to an organism of that type.

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# THE RESOLUTION OF ALPHA-SUBSTITUTED PYRROLIDINES[1]

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Attempts to discover a quantitative connection between rotatory power and chemical constitution of an active molecule have been numerous since Crum Brown[2] and Guye [3] published the theory connecting certain constants representing the radicals with molecular rotatory power. Their calculations of rotations from the radicals attached to the asymmetric carbon atom proved to be unsuccessful even for homologous series. Betti[4] and Rule[5] obtained very good correlations in some series of compounds when they considered the electrical properties of the radicals as masured by dissociation constants and dipole moments. Betti compared the rotations of aldehydo-aminic derivatives of $\beta$-naphtholphenylamino methane $C_6H_5-\underset{C_{10}H_5OH}{\overset{H}{C}}-N=\overset{H}{C}-R$, with the dissociation constants of the acids, RCOOH, where R is a substituted phenyl radical. Rule compared rotations of a series of homogeneous 1-menthyl esters of mono-substituted acetic acids, $XCH_2$—COOH, with the dissociation constants of the acids. According to these investigators increasing the "electronegativity" of one of the four radicals attached to the asymmetric carbon atom increased the molecular rotation. Levene [6] found that in the derivatives of disubstituted acetic acids, $R_1-\underset{R_2}{\overset{H}{C}}-COOH$, the direction of the rotation and the respective numerical values of the latter, might be regarded as functions of the polarity of the substituting group. From these results it can be seen that the electrical properties of substituents exert a marked influence on the rotary power of optically active compounds.

To initiate a study of the effect on the rotatory power due to varying the radical, R, attached to the asymmetric carbon atom in a series of $\alpha$-substituted pyrrolidines, the compounds were prepared and resolved. The preparations were accomplished according to a general method developed by Craig, Bulbrook and Hixon[7]. $\alpha$,p-Chlorophenylpyrrolidine and the corresponding pyrroline were prepared for the first time; $\alpha$,n-butylpyrrolidine and the corresponding pyrroline were prepared by this method

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[1] Original thesis submitted December, 1935. Doctoral thesis number 350.

[2] Crum Brown, *Proc. Roy. Soc., Edinburgh*, **17**, 181. 1890.

[3] Guye, *Compt. rend.*, **110**, 714. 1890.

[4] Betti, *Gazz. chim. ital.*, **50**, II, 276. 1920.

[5] Rule, *J. Chem. Soc.*, **125**, 1121. 1924.

[6] Levene, *J. Biol. Chem.*, **84**, 571. 1929.

[7] Craig, Bulbrook and Hixon, *J. Am. Chem. Soc.*, **53**, 1831. 1931.

for the first time. α-Phenylpyrrolidine, α-ethylpyrrolidine, α,p-tolylpyrrolidine and α-cyclohexylpyrrolidine were prepared. They were resolved for the first time into dextro and laevo forms. Dextro tartaric acid was the resolving agent for the d-base and laevo-tartaric acid for the l-base of the first three compounds mentioned. Dextro camphoric acid was used to obtain both the d- and l-forms of α-cyclohexylpyrrolidine. Attempts at resolving α,p-chlorophenylpyrrolidine, α,n-butylpyrrolidine and α-benzylpyrrolidine were unsuccessful. Micro Dumas nitrogen analyses were made on all new compounds, and others were characterized by boiling points or melting points and derivatives. The densities and refractive indices of the bases were determined. Rotations of the pure pyrrolidines and of ethyl alcohol solutions of the pyrrolidines were measured with a sodium arc as the source of light. Rotations of alcohol and of water solutions of the salts of the active bases were read.

If comparisons of rotatory powers are to be made it is necessary to compare values for active forms having the same configuration. d,α-Phenylpyrrolidine was reduced by hydrogenation under pressure in the presence of platinum oxide platinum black catalyst. The product obtained from this reduction was proved to be identical with the d,α-cyclohexylpyrrolidine obtained from the resolution of the racemic mixture with d-camphoric acid. Thus d,α-cyclohexylpyrrolidine and d,α-phenylpyrrolidine have the same configuration. It is assumed that the dextro forms of α-ethylpyrrolidine and α-cyclohexylpyrrolidine have the same configuration as the dextro forms of α-phenylpyrrolidine and α-cyclohexylpyrrolidine.

When the radical, R, is varied the molecular rotation of the liquid active α-substituted pyrrolidines varies. Changing R from a relatively "electropositive" group, cyclohexyl, to a relatively "electronegative" group, phenyl, changes the rotatory power from + 14.38 at 25° to + 103 at 25°. The other two members of the series show molecular rotations between these two values.

The influence exerted by R on the molecular rotatory power is compared with the effect on the dissociation constants and with similar variations in a series of α-substituted ethylamines. When the dissocciation constants of these compounds in methanol and in water and the dissociation constants of the corresponding primary amines, R—$NH_2$, are arranged in decreasing order of magnitude, the rotatory powers of the α-ethylamines and α-substituted pyrrolidines with the exception of the α-ethylpyrrolidine, are found to fall in increasing order of magnitude. An increase in the dissociation constants of the corresponding acids, R—COOH, corresponded to an increase in the molecular rotations of the active compounds.

Burch[8] determined the values for the dissociation constants of the α-ethylamines in methanol which are nearly equal to the values obtained for the α-substituted pyrrolidines. In comparing the rotatory powers of the two series a large difference in the values for the molecular rotations having like R substituents is observed. The presence of the ring in the α-substituted pyrrolidines seems to influence the rotary power much more than it does the dissociation constant. The configurations in the two

[8] Burch, unpublished thesis for Ph. D. Iowa State College Library. 1935.

series are also dissimilar. In the α-substituted ethylamine series Leithe[9] converted α-phenylethylamine into α-cyclohexylethylamine by catalytic hydrogenation and showed that they had opposite signs of rotation for the same configuration. The dextro form of α-phenylpyrrolidine was reduced to the dextro form of α-cyclohexylpyrrolidine showing that the sign of rotation remains the same.

It is concluded that some relationship exists between the optical rotatory power of the α-substituted pyrrolidines and the electrical property of the radical, R, as measured by dissociation constants and that the relation is even more complex than the one exhibited by the α-ethylamines.

[9] Leithe, *Ber.*, 63, 800. 1930.

# A STUDY OF 2,3 BUTYLENE GLYCOL AND ITS DERIVATIVES[1]

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Recognition of their usefulness and extreme versatility has brought about an ever increasing production of organic compounds. New compounds are constantly being developed to meet specific needs and new uses are being discoverd for products already known. The sources of raw materials for the synthesis of these compounds may be divided into two classes: the organic deposits in the earth, the amount of which, though large, is limited, and the practically inexhaustible supply of agricultural products. The gradual depletion of the first source will force a turn to the greater utilization of the latter. Because of this, and because a greater production and more varied use of agricultural produce will satisfy the long-felt economic need for more stable agricultural prices, it seems expedient to try to enlarge our knowledge of the fundamental compounds obtainable from plants, so that we can produce and apply them most efficiently. 2,3 Butylene glycol is such a compound and the object of this study was to add somewhat to the information concerning it.

Though 2,3 butylene glycol was first isolated from a fermentation mixture in 1906 (1), and small amounts had been synthesized by a variety of methods prior to that date, it was not until recently that much attention was given to its possibilities as a useful industrial chemical. However, the glycol is now being produced on a semi-commercial scale and is available at a moderate price. In these laboratories, in 1934, Kendall (2) developed the conditions for maximum conversion of sucrose to 2,3 butylene glycol by fermentation and this thesis is intended to supplement that work by presenting some of the properties of the glycol and its derivatives.

With the help of Kendall's findings, nearly 2600 g. of 2,3 butylene glycol were synthesized biochemically. Recovery from the beer was effected by means of continuous ether extraction. A diagram of the extraction apparatus and a brief discussion of the problems involved are given. The vapor pressure of the glycol was determined at several temperatures and shown to be fairly low, even at 100° C.

The preparation, in many instances by several methods, of the following derivatives of 2,3 butylene glycol is described, and the boiling-points, densities, and refractive indices of these compounds reported: 2,3 butylene bromohydrin, 2,3 butylene chlorohydrin, 2,3 butylene dibromide, 2,3 butyline dichloride, 2,3 butylene glycol monomethyl ether, 2,3 butylene glycol diethyl ether, 2,3 butylene glycol mono-isoprpyl ether, 2,3 butylene glycol di-n- propyl ether, 2,3 butylene glycol mono-iso-propl ether, 2,3 butylene glycol mono-n-butyl ether, 2, 3 butylene glycol di-n-butyl ether, 2 bromo 3 methoxy butane, 2 bromo 3 ethoxy butane, 2 chloro 2 methoxy butane,

[1] Original thesis submitted July, 1935. Doctoral thesis number 333.

2 chloro 3 ethoxy butane, 2,3 butylene glycol diactetate, 2,3 butyline glycol mono-methyl ether acetate, and 2,3 butylene diamine.

Because of its theoretical importance as a fundamental compound and the comparatively little information available concerning it, considerable attention is devoted to 2,3 butylene diamine. The substance was synthesized by many methods, including those of previous workers, and the conclusion drawn that reduction of dimethyl glyoxine in alcoholoc hydrochloric acid, using platinum black as the catalyst, is the most expedient, though reduction of the glyoxine in acetic anhydride would be much better if the resulting acetyl derivative were more readily hydrolyzed. Gradual addition of hydrochloric acid during the reduction, instead of all at the beginning, was found to increase the yield. A diagram of a flask developed for these reductions, as well as graphs for the reductions in glacial acetic acid and in acetic anhydride, are shown.

An electrometric titration of the diamine was run and the dissociation constants calculated from the data obtained. They are (at 25° C.): $K_1 = 5.5 \times 10^{-5}$! $K_2 = 2.9 \times 10^{-8}$. The preparation and melting points of several derivatives characterizing the diamine are given.

The 2,3 butylene diamine prepared was resolved by means of d*a*-bromocamphor $\pi$ sulphonic acid. The salt of the dextro isomer was obtained quite pure, but that of the laevo form could not be crystallized. The values found for the optical rotation of the two forms were $[\alpha]^{25}_D = +7.3$ and $-5.0°$, the latter figure being for a 10 per cent aqueous solution. Strack and Schwaneberg (3) resolved the diamine just recently, using tartaric acid, and obtained a value of $[\alpha]^{18}_D = \pm 4.8°$ for a 5 per cent aqueous solution. Dextro 2,3 butylene glycol, $[\alpha]^{25}_D = +6.9°$ was prepared from the dextro diamine and was in turn converted to laevorotary 2,3 butylene dichloride $[\alpha]^{25}_D = -15.36°$.

A discussion of the maximum possible yield of 2,3 butylene glycol biochemically obtainable from raw materials is presented, together with a proposed mechanism of reaction.

The properties of the derivatives of 2,3 butylene glycol fall midway between those of the simple monohydric alcohols and glycerol. They are very similar to the properties of ethylene glycol, but exhibit a little more of a hydrocarbon nature. Many of the butylene glycol derivatives are excellent solvents and might find application in cases in which their specific properties such as boiling-point, volatility, and viscosity, would make them more desirable than similar derivatives of glycerol or ethylene glycol. If the present day manifold uses of glycerol and ethylene glycol were examined, many instances undoubtedly would be found in which 2,3 butylene glycol would be superior, and many other cases in which the butylene glycol would be an admirable substitute if the price of glycerol or ethylene glycol should get out of bonds, as was true during the World War.

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# THE ANIMAL PARASITES OF THE WOODCHUCK (MARMOTA MONAX L.) WITH SPECIAL REFERENCE TO PROTOZOA[1]

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The present investigation was undertaken in order to secure more definite data on the morphology, life histories and other biological factors regarding the protozoa of the common woodchuck, *Marmota monax* Linn., and to make a complete record of all animal parasites known to live on or within that host species. The genus Chilomastix Alexeieff has been given special consideration among the protozoa, since many factors regarding morphology, method of division, and binuclearity in both trophozoites and cysts are not well understood. Further considerations have been given *Trichomonas digranula* Crouch and *T. wenrichi* Crouch. New species of Chilomastix and Endamoeba have been described.

## MATERIALS AND TECHNIQUES

Parasites were collected from 56 woodchucks; 35 from the vicinity of Ames, Iowa, and 21 from Franklin County, Kentucky. Cover glass smears were made from the intestinal content fixed in Schaudinn's fluid, and stained according to Heidenhain's iron-alum hematoxylin method. Delafield's hematoxylin was also used, but did not give very good results.

## THE PROTOZOAN PARASITES

*Chilomastix instabilis*, n. sp., is morphologically similar to other species of the genus. There are four or six blepharoplasts anterior to the nucleus, to which the three anterior flagella, cytostomal flagellum, parabasal body, and parastyle are attached. These blepharoplasts are connected by short fibrils. Becker's parabasal body is found in the majority of specimens, but it is not known whether it is attached to blepharoplasts or to other structures. The cystostmal flagellum is not attached to an undulating membrane. The average size of this species is $12.1\mu \times 9.45\mu$.

The cysts of *C. instabilis* are typically egg-shaped. The average size is $9.45\mu \times 8.19\mu$. No intracystic mitosis or binucleate cysts have been observed. Binucleate trophozoites are fairly common, and it is probable that binucleate cysts result from encystment of these forms.

The process of division in *C. instabilis* is different in some respects from other species. The blepharoplasts do not enter in the formation of the mitotic spindle during division. They remain in their normal positions at the anterior end of the body and ultimately degenerate. A centrosome, situated on or near the anterior margin of the nucleus, becomes the division center. None of the organelles associated with the blepharoplasts migrate with the nucleus during division. New sets of organelles are produced from the young blepharoplasts at the ends of the mitotic spindle.

[1] Original thesis submitted June, 1936. Doctoral thesis number 368.

Approximately eight chromosomes appear during the early stages of division.

The check list of the genus Chilomastix includes 24 names of species exclusive of *C. instabilis,* and 26 records in which the species was not designated. The total number of species is reduced to 16 through synonomy and homonomy. Fifty-nine host records are listed.

*Endamoeba marmotae* n. sp., is similar to other *E. coli*-type *endamoebae.* The average size of mononucleate trophozoites is 16.56$\mu$ x 14.11$\mu$; trophozoites with more than one nucleus are usually larger. The endoplasm and ectoplasm are poorly differentiated. The pseudopodia are finely granulated. The cysts measure 12$\mu$ in diameter, on an average. The cyst wall is about 0.5$\mu$ thick. Eight nuclei occur in the fully mature cysts. The chromatoid bodies are rather large and rod-shaped.

*Trichomonas digranula* appears to be a primitive member of the genus. The "adult" of this species is similar to early division stages in other species. The connecting rhizoplast between the blepharoplasts becomes functional as the paradesmose during division.

Binucleate trophozoites of *T. digranula* have a striking similarity to some members of the genus Hexamita. It is suggested that both the highly developed members of the genus Trichomonas and the hexamitids probably arose from a primitive ancestral trichomonad similar to *T. digranula.*

*Trichomonas wenrichi* has a life history similar to most other reported species. A new chromatic basal rod grows from the blepharoplasts before any other apparent changes occur. The prophase chromosomes are constricted in the middle, and appear to be composed of two nearly spherical masses. There are six chromosomes in this species. The karyosome is visible only during the organization of the prophase chromosomes. The parent axostyle does not completely degenerate during division. From the fact that one of the daughter axostyles is usually larger than the other, it seems probable that the old axostyle contributes in some way to the production of the larger daughter axostyle. The parent chromatic basal rod is retained in one of the daughter trophozoites.

Somatomy, or fragmentation of the cytoplasm, is noted in *T. wenrichi* and other trichomonads. Globules of cytoplasm are pinched off from the posterior end of the body. Some of these globules contain undigested materials and Sphaerita parasites, while others contain no solid matter. Somatomy appears to serve as a method of eliminating waste materials. It also serves as a method of overcoming hyperparasitism. From the fact that metabolic products are probably eliminated in this manner, somatomy may function as rejuvenation process.

## HYPERPARASITISM

Sphaerita parasites are found in all species of protozoa from the common woodchuck, with the exception of the coccidia and Hexamita. At least two species of Sphaerita are present. These hyperparasites do not appear to be highly pathogenic to their parasite host.

The following species of protozoa are recorded from the common woodchuck, *Marmota monax*: MASTIGOPHORA—*Chilomastix instabilis,* n. sp., *Trichomonas cryptonucleata* Crouch, *T. digranula* Crouch, *T. marmotae* Crouch, *T. wenrichi* Crouch, *Hexamita marmotae* Crouch; RHIZOPODA—*Endamoeba marmotae* n. sp.; SPOROZOA— *Eimeria*

*monacis* Fish, *E. os* Crouch and Becker, *E. perforoides* Crouch and Becker.

PARASITES EXCLUSIVE OF THE PROTOZOA

NEMATHELMINTHES: *Ascaris laevis* Leidy, *Citellina marmotae* Manter, *Citellinema monacis* Manter. ARTHROPODA: Inecta-*Cyclophthirus (= Enderleinellus) marmotae* (Ferris), *Oropsylla arctomys* (Baker), *Opisodasys pseudoarctomys* (Baker), *Orchopeas wickhami* (Baker), *Ctenocephalides canis* (Curtis), *Ixodes hexagonus* Pack., *Dermacentor variabilis, Atricholaelaps glasowi* (Ewing), *Ichoronyssus sternalis* Ewing, *Trombicula blarinae* Ewing.

# A TOXICOLOGICAL INVESTIGATION OF NICOTINE ON THE GOLDFISH AND THE COCKROACH

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## PART I

## THE EFFECT OF SOLUTIONS OF THE BASE AND SULPHATE ON THE GOLDFISH, CARASSIUS AURATUS (L.)[1]

The difference in the physiological action exhibited by the molecules and the ions of various alkaloids, when used as anesthetics and insecticides, has attracted the attention of a number of research workers. Most of the entomologists who first investigated nicotine believed that the greater toxicity of the undissociated molecule resulted from its greater volatility. This theory, however, did not fully explain the difference in toxicity, for it was shown that there was still a marked difference when certain experimental animals were exposed to solutions containing largely nicotine molecules and others to solutions largely of dissociated nicotine.

According to the results obtained by other investigators, the free base of most alkaloids exhibits a more marked toxic action, when applied to the external surface, than solutions of the salt of the same molar concentration. However, it appears that conclusive evidence has not been presented which fully explains the cause of this phenomenon.

It was thought that additional light could be thrown upon the subject by approaching the matter from a quantitative basis. Consequently, quantitative determinations were made of the amount of nicotine in the goldfish, *Carassius auratus,* after death in solutions of the free base and in solutions of the sulphate. In addition, quantitative determinations were made of the speeds of penetration of nicotine base and of its sulphate into the body. The quantity of nicotine that the goldfish could tolerate without lethal effects, and the distribution of nicotine (from solutions of the base and sulphate) in the various tissues of the organism were also investigated.

A review of the literature was made, and 33 of the more pertinent references were discussed in the thesis. The source and care of the biological material and the chemical composition of the materials used in the investigation were given. Also, the method of handling the goldfish, exposing them to the test solutions, extracting the nicotine from the tissues and recovering it, and determining the quantity of nicotine in the test animals were discussed in detail. In addition, the criterion of toxic effect, the endpoint, was given.

In the first tests, the goldfish were exposed until death in solutions of nicotine base and of the sulphate of various pH values at concentrations of 0.002 M, 0.001 M, and 0.0002 M. At the time of death of the animals, the

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[1] Original thesis submitted December, 1935. Doctoral thesis number 353.

quantity of nicotine recovered was approximately the same regardless of the pH of the solution in which they were exposed. For example, a mean of 0.034 mg./g. (milligram per gram of body weight) of nicotine was present in the bodies of the fish killed in solutions of 0.001 M nicotine base; whereas those killed in solutions of the sulphate at pH values of 7.3 and 5.0 showed a mean of 0.033 mg./g. and 0.034 mg./g., respectively. In solutions of the same molar concentration of nicotine, the free base penetrated much more rapidly than the partly ionized or completely ionized nicotine sulphate. In nicotine solutions of 0.002 M, the mean time to death was 102 seconds for the free base and 174 seconds, 355 seconds, and 1,141 seconds for the nicotine sulphate at pH values of 7.5, 5.0, and 2.6, respectively. Therefore, it is evident that the rate of entrance of the nicotine, hence the speed of toxic action, decreases with the increase in the ionization of the nicotine molecules.

The nicotine is not entirely absorbed in the skin, but a considerable quantity penetrates the deeper lying tissues. This was demonstrated by analysis of muscle tissue, integument, fins and gills taken from fish exposed to solutions of nicotine.

The survival time of fish when exposed to solutions of 0.002 M nicotine base was determined. It was found that 50 percent of the population failed to recover after being exposed for about 23 seconds.

Charts and tables of the experimental data are given in the thesis.

## PART II

### THE EFFECT BY INJECTION OF SOLUTIONS OF THE BASE AND SULPHATE INTO THE COCKROACH, PERIPLANETA AMERICANA (L.)

It was shown in the investigation on the goldfish that the greater toxicity of the free base of nicotine was a result of its more rapid speed of entrance into the organism. If the problem of penetration through the integument was eliminated by placing the toxic solution in direct contact with the blood, it seemed likely that the true relationship between the toxicity of the ions and of the molecules of nicotine could be demonstrated experimentally.

Accordingly, experiments were conducted in which nicotine solutions of known pH values were injected into the body cavity of the experimental animal, which was the American cockroach.

A review of the literature pertaining to injection of alkaloids into insects was made. The references are given in the thesis.

The apparatus for injection, and also the method of injecting the compound into the roach were described. Two criteria of toxic action were used in this investigation: one was death and the other was the period of time which elapsed from injection to complete paralysis and from injection to recovery from paralysis of the various pairs of legs.

In all cases 0.05 cc. of solution was injected, the temperature of which was 23° C.

In the first experiment molar concentrations of 0.05, 0.02, 0.013, and 0.01 nicotine base and sulphate were injected. The pH values of the sulphate were approximately 7 and 3. When death was the criterion of toxic action, it was found that at any given molarity of nicotine there were

no significant differences in the percentage of mortality of the roaches injected with the base and those injected with the sulphate.

Tests were also made with 0.01 M nicotine base and the sulphate at pH values of 6.7 and 2.8. In these tests, where the time from injection to paralysis and recovery of the pairs of legs was taken as the criterion of effect, again there were no significant differences in the toxicity of the free base and the sulphate.

These investigations on the goldfish and on the cockroach seem to point to the fact that the difference in toxicity of nicotine and nicotine sulphate is the result of the greater speed of penetration of the molecules. For, when the toxic effect is dependent upon the penetration of a membrane (body covering of the goldfish), the free nicotine is much more toxic than solutions of of the sulphate of the same nicotine concentration; whereas, when solutions of either the ionized or molecular nicotine are injected directly into the cavity of the body (cockroach) there is no difference in toxic action.

Charts and tables of the experimental data are given in the thesis.

# THE INFLUENCE OF VARIOUS PROCEDURES ON THE FLAVOR AND KEEPING QUALITY OF BUTTER[1]

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Some of the constituents which are responsible for the desirable flavor and aroma of butter cultures have been studied in considerable detail. The work presented in this thesis was carried on in an attempt to determine the best methods of using culture in order to transfer its desirable qualities to the resulting butter.

The cream used was gathered cream of varying quality. In most of the comparisons sweet cream was employed, but in a few cases comparisons were made using sour cream.

The scores on the butter were treated statistically by the method suggested by Brandt (1) in order to determine whether the differences in score were highly significant, significant or not significant.

## THE INFLUENCE OF THE METHOD OF USING BUTTER CULTURE ON THE FLAVOR AND KEEPING QUALITY OF BUTTER

The method of using butter culture was found to have considerable influence on the flavor and keeping quality of butter. The addition of 8 per cent butter culture to pasteurized and cooled cream 16 hours before churning resulted in butter significantly higher in score than the addition of 8 per cent culture at the time of churning; this was the case when the butter was fresh and also after cold storage, and occurred with both sweet and sour cream. Holding the pasteurized and cooled cream containing 8 per cent culture at a low temperature for 16 hours before churning had little effect on the acidity of the cream but did result in an increase in its acetylmethylcarbinol plus diacetyl content. Holding periods longer than 16 hours did not give appreciable improvements in the scores of the resulting butter. In some cases the acetylmethylcarbinol plus diacetyl content of the cream was increased during such holding periods but more often a decrease took place.

When 8 per cent butter culture was added to pasteurized cream that had been cooled to 70° F. and the cream ripened for 1 hour, the butter was more often high in score than the butter made by the addition of 8 per cent culture to pasteurized and cooled cream. The differences in score were not significant when the butter was fresh and after cold storage, but were significant after the butter had been held a few weeks at about 28° F. The ripening of the cream for 1 hour at 70° F. usually brought about an increase in the acetylmethylcarbinol content of the cream but not in the acidity.

Three methods of using culture gave butter which did not differ significantly in score when fresh, after holding a few weeks at 28° F. or after cold storage. These methods were: (A) the addition of 8 per cent

[1] Original thesis submitted June, 1936. Doctoral thesis number 382.

culture to pasteurized and cooled cream followed by holding at 28° to 36° F., (B) the addition of 8 per cent culture to pasteurized and cooled cream with holding at 41° to 52° F., and (C) the addition of 8 per cent culture to pasteurized cream at 70° F. followed by ripening for 1 hour, cooling and holding at 28° to 36° F. With methods A and B the acetylmethylcarbinol plus diacetyl contents were about equal and both methods resulted in higher acetylmethylcarbinol plus diacetyl contents than method C.

Butter made by the addition of 8 per cent culture to pasteurized and cooled cream was usually significantly higher in score than butter made without culture when scored fresh and also after holding a few weeks at about 28° F.; after cold storage there was commonly little difference in the scores. This was true with both sweet and sour cream. There was usually very little or no acetylmethylcarbinol plus diacetyl in the cream churned without culture.

### THE INFLUENCE OF THE TYPE OF BUTTER CULTURE ON THE FLAVOR AND KEEPING QUALITY OF BUTTER

A modified culture was made as follows: *Streptococcus paracitrovorus* was grown in pasteurized milk at 70° F. for 24 hours; the milk was than acidified with 0.3 per cent sulfuric acid and 0.15 per cent citric acid, held another 24 hours at 70° F. and finally cooled to 40° F. With either sweet or sour cream, the use of this modified culture resulted in butter significantly higher in score than the use of regular culture, or the use of no culture, when the butter was scored fresh or after holding a few weeks at about 28° F.; after cold storage there was no significant difference in the scores.

Trials were made using regular and modified culture neutralized back to 0.3 per cent acid. The object in neutralizing the cultures was to attempt to retain the desirable flavors resulting from the use of culture without developing a sour flavor in the butter. The neutralized cultures did not give butter significantly higher in score than the unneutralized cultures when scored fresh, after holding a few weeks at about 28° F. or after cold storage. The use of either regular or modified culture that had been neutralized gave butter significantly higher in score than the use of no culture when the butter was fresh and also after holding a few weeks at about 28° F.; after cold storage there was not a significant difference in the scores.

It was noted that in some instances the desirable flavor imparted to butter by the use of butter culture disappeared on holding. Since butter cultures that are held for extended periods commonly show a decrease in the acetylmethylcarbinol plus diacetyl contents, as a result of the action of butter culture organisms, an attempt was made to prevent this destruction of the desirable flavor in butter by pasteurizing the ripened butter culture. The curd was filtered off and the filtrate added to the cream. However, butter made using regular culture was significantly higher in score than the butter made using pasteurized culture when scored fresh or after holding a few weeks at about 28° F.; after cold storage there was no significant difference in the scores.

### THE INFLUENCE OF THE ADDITION OF ACETYLMETHYLCARBINOL AND DIACETYL TO BUTTER ON ITS FLAVOR AND KEEPING QUALITY

Butter made by the addition of diacetyl was not significantly different in score than butter made with butter culture when scored fresh, after holding a few weeks at about 28° F. or after cold storage. The addition of *Streptococcus lactis* or *Streptococcus paracitrovorus* to cream and acetylmethylcarbinol to the resulting butter did not give butter significantly different in score than butter made with butter culture when scored fresh, after holding a few weeks at about 28° F., or after cold storage.

### THE MANUFACTURE OF HIGH SCORING BUTTER

The results indicate that in the manufacture of butter higher in score than any of the commercial grades the flavor of the cream and its subsequent treatment, with respect to neutralization and use of butter culture, are of extreme importance. The practices found of value were the addition of 0.15 per cent citric acid to milk used for butter culture, and the neutralization of sweet cream after pasteurization to 0.10 per cent acid before adding the culture.

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# ON THE PENETRATION OF CERTAIN ARSENICAL COMPOUNDS INTO THE BODY OF THE AMERICAN COCKROACH, PERIPLANETA AMERICANA (L.)[1]

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Arsenical compounds have been used as insecticides for many years, yet little attention has been given to their effect as contact poisons.

This work was undertaken to determine the nature of the penetration of certain arsenical compounds through the integument of the adult American cockroach, *Periplaneta americana* (L.), when applied as a dry powder, and to learn to what extent such compounds became distributed in the insect body after penetration had taken place.

A comparison was made of the penetration and distribution of arsenious oxide and sodium arsenite.

Application was made by sealing the dry powdered toxicant in a wax cell on the metathoracic tergite of the roach. Entire roaches as well as parts and tissues were tested by means of a modified Gutzeit method to determine the amount of arsenic recoverable. The following are modifications of the method described in A. O. A. C. (1930). The generator tube was made as one unit and a paraffined cork stopper was substituted for the rubber stoppers. The initial charge in the Gutzeit generator was made up of three grams of 20 mesh granulated zinc, one millimeter of hydrochloric acid—stannous chloride solution, and 2.5 milliliters of distilled water. Also no potassium iodide was used. Instead of an aliquot the entire sample was used. The percentage of mercuric bromide was varied from 1.5 to nine. After digestion the sample was made up to nine grams with sulphuric acid. Results were recorded by means of paper strips sensitized with mercuric bromide and measured in milligrams of arsenious oxide.

Each time a series of determinations was made on solutions containing an unknown amount of arsenic, a series of determinations was also made with a number of dilutions from a standard arsenic solution made up from arsenious oxide, that would exceed any amount which might be found in the unknown. A standard graph was constructed by plotting on cross-sectioned paper the averages of the measurements from the standard arsenic solutions. Thus all results were read directly in milligrams of arsenious oxide.

Experiments were designed to determine the effect on penetration of increased area available for penetration and of varying the amounts, by weight, of the powdered arsenious oxide. When the area of the application cell was doubled the average amount of arsenic present in the insect body after 72 hours was 0.016 mg./g. body weight. In the case of cockroaches treated with the standard cell (3.2 mm. in diameter) the average

[1] Original thesis submitted June, 1936. Doctoral thesis number 388.

amount recovered was 0.006 mg./g. body weight. The difference between these two means is significant.

In experiments in which the amount of arsenious oxide was varied, the average amount of arsenic recovered after 72 hours was 0.006 mg./g. body weight when 0.01 gram was used in the cell and 0.007 mg./g. body weight when 0.03 gram was used.

A comparison was made between the penetration of arsenious oxide and that of sodium arsenite. The average amount of arsenic recovered from the bodies of cockroaches treated with arsenious oxide was, after 72 hours, 0.010 mg./g. body weight; after 120 hours, 0.025 mg./g. body weight; after 168 hours, 0.021 mg./g. body weight. With sodium arsenite the amounts were as follows: after 120 hours, 0.103 mg./g. body weight; after 168 hours, 0.162 mg./g. body weight. Sodium arsenite penetrates the integument faster than arsenious oxide.

These two toxicants vary markedly in their solubility in water. Arsenious oxide is moderately and very slowly soluble in water, Anderson and Story (1923). It is increasingly soluble in alkaline solutions due to the formation of soluble arsenites. Sodium arsenite is very soluble in water. These compounds seem to follow the same order of solubility in a fluid present on the integument of the cockroach. The arsenious acid powder became dampened after seven days in the cell and adhered to the integument. The sodium arsenite powder became dampened after a few hours and by the end of seven days became largely dissolved in the liquid present. Apparently penetration does not take place until the dry powder has become dissolved.

Results show definitely that arsenic will penetrate through the integument of a cockroach when applied either as arsenious oxide or as sodium arsenite.

Finally a comparison was made of the distribution of arsenic in parts and tissues of the adult when applied as arsenious oxide or sodium arsenite.

Groups of five cockroaches were used for dissection purposes. Tissues and parts from such a group were weighed in tared weighing bottles before they were transferred to Kjeldahl flasks for digestion.

The average weight of tissues and parts was as follows: integument 0.235 gram; legs 0.228 gram; wings 0.016 gram; digestive tract 0.079 gram; thoracic muscle 0.090 gram; central nervous system 0.005 gram.

When rather high concentrations are built up in the insect body arsenic may be recovered in all parts and tissues. Such concentrations were found when sodium arsenite was employed as the toxicant. When the less soluble arsenious oxid was used the distribution of the arsenic was practically limited to the digestive tract and to parts and tissues near the point of application.

Cockroaches which had been treated for 72 hours with arsenious oxide had an average of 0.002 mg. of arsenic in the voided feces. This, together with the fact that the largest quantity of arsenic was recovered from the digestive tract, shows that arsenic is eliminated to a considerable extent by means of the digestive tract.

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# AN INVESTIGATION OF THE PENETRATION OF PYRIDINE, PIPERIDINE AND NICOTINE INTO THE BODIES OF INSECTS[1]

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The problem of relative toxic efficiency of various compounds to animals has been the concern of many investigators. Among invertebrates, very few studies have followed the course of entrance of poisons into the entire body or into the tissues. This inquiry attempts to establish penetration of pyridine, piperidine, and nicotine into the body of an insect from saturated atmospheres of their vapors in air. Information is also presented on the speed of entrance and the quantity of the compounds which enter the body within a given time. Some facts are presented concerning the distribution of the compounds in the issues.

## MATERIALS

The insects used in the investigation were adults and nymphs of the American cockroach, *Periplaneta americana* (L.) and late instar larvae of the corn ear worm, *Heliothis obsoleta* Fab. The toxic compounds employed in the experiments were pyridine, redistilled from a reagent grade and collected between 112° and 113° C. (740 mm.); piperidine, C.P. (Eastman Kodak Co.), and nicotine, redistilled (99.32 per cent).

From a large number of reagents giving color reactions or precipitates with the three toxic compounds, three were selected which provided two independent tests for each of the three toxic compounds. They were Wagner's reagent, phosphomolybdic acid reagent and gold bromide reagent. Wagner's reagent detects pyridine at 0.004 gram per 100 cc. or less. Phosphomolybdic acid reagent was used to precipitate all three of the compounds. With piperidine it gives a crystalline precipitate discernible at 0.05 gram per 100 cc., and a turbidity with nicotine at 0.002 gram per 100 cc. Gold bromide reagent confirmed nicotine and piperidine at lower concentrations.

The sensitivities of various reagents were determined with aqueous solutions of the pure compounds acidified with tartaric acid. Known solutions of the compounds were tested by precipitation with the reagent. If precipitation resulted, the solution was diluted by one-half with distilled water and successively by half steps until a concentration was reached which gave a faint but unmistakable turbidity. This concentration was accepted as the limit of sensitivity of the particular compound, and it furnished the factor used in calculating the quantity of a toxic compound in the extract derived from the treated insects.

## METHODS

The insects were exposed to the vapors in an ordinary gas bottle of 250 cc. capacity closed with a cork stopper. Inserted into the cork was a

[1] Original thesis submitted June, 1933. Doctoral thesis number 249.

hook from which a wire cage for the insects was suspended. An excess of the liquid compound was placed in the bottle, assuring practically complete saturation of the air around the insects. Exposure times varied with the compounds, to include ranges of no, slight and extreme mortalities. The temperature for exposure was 30° C. for the cockroaches and room temperature for corn ear worm larvae.

Since pyridine, piperidine and nicotine are volatile in steam, a steam distillation method was used to extract the compounds from the tissues. After removal from the exposure flask the insects were washed in a stream of distilled water and refluxed for 10 minutes in a Kjeldahl flask with 20 cc. absolute alcohol, acidified with tartaric acid. The alcohol was evaporated in a current of air and the syrupy residue was dissolved in 20 cc. of distilled water. This acid aqueous extract was made alkaline with dilute potassium hydroxide solution and steam was passed through the alkaline extract until 100 cc. of the distillate were collected. The distillate, acidified with tartaric acid, was reduced to 10 cc.

In determinations of various tissues or parts, the tissues were dissected out into tared weighing bottles. The muscular tissue was obtained chiefly from the leg and wing muscles; the digestive tract included the whole alimentary canal; the nerve tissue included the entire ventral nerve cord but not the brain. The fat body tissue was removed directly from the body cavity. The cuticula was the entire exoskeleton with as much as possible of the abdominal muscles and fatty tissue removed. In experiments with *H. obsoleta*, time and material permitted only the determination of pyridine in the blood. A short extraction method was used.

The standard volumes of distillates from the steam distillation of entire insects and insect tissues were diluted quantitatively and tested with an adequate reagent to determine the concentration of the toxic compound in the distillate. The quantitative values are of greater worth as comparative measures than as absolute measures of the compounds present in insects for the reagents were not necessarily employed at the exact limits of detection, but at "end-points" at which detection was certain and comparison could be made without great difficulty.

## DISCUSSION

The concentration of pyridine in the bodies of cockroaches after 51 minutes when half the number was dead was 2.9 mg./g. Pyridine seemed to exhibit no great selectivity for any part or tissue. However, large quantities were found in the ventral nerve cord. Much less pyridine was present in the cockroach up to 60 minutes than in the blood of the corn ear worm, which had approximated a concentration of 11 mg./g. after 95 minutes.

About 50 per cent of the roaches had died after 12.5 minutes when the body contained a piperidine concentration of 1 mg./g. The greater accumulation of piperidine went to the muscle, cuticula and ventral nerve cord. This points to the importance of penetration through the cuticula, tissues nearest the cuticula containing the greatest quantity of piperidine. A gradient is established from the cuitcula to the innermost tissues.

The nicotine concentration at which 50 per cent of the roaches had been killed was about 1.2 mg./g. and the exposure time was 14.3 hours.

Nicotine steadily accumulated in the cuticula even after the concentrations in the digestive tract and nerve tissues had reached equilibrium.

In 12.5 minutes the amount of pyridine in the body is comparable to that of piperidine, while a much smaller concentration of nicotine has been attained. Piperidine has killed 50 per cent of the insects, pyridine and nicotine, none. The rate of entrance of nicotine may be slower because the outside concentration is relatively very low, the amount vaporized penetrating as it becomes available.

Considering toxicity as a function of outside concentration as well as the concentration in the tissues and of the time, the relative toxicity of the three compounds in decreasing order is nicotine > piperidine > pyridine.

# INVESTIGATION OF CODLING MOTH POPULATIONS AS THEY AFFECT CONTROL EXPERIMENTS[1]

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The chief purpose of this investigation was to improve the Iowa orchardists' defense against the codling moth. In this connection attention was given to the technique of codling moth field experimentation which would not only facilitate this purpose, but also furnish information of value to other workers in the field.

As many as fifty or more factors may affect the population of codling moth on a tree at harvest. From this array the worker must select one or two for his experiment, attempting at the same time to keep the remaining variables as nearly constant as possible. That all variation can not be eliminated is obvious; therefore the worker must so replicate the units of his experiment that the uncontrolled variation may be estimated and his results relied upon proportional to the size of this random error.

The results of a banding experiment at the experimental orchard in Mitchellville, Iowa, indicate that more worms are caught on trees bearing bands on the scaffold branches in addition to one on the main trunk than on trees singly banded on the main trunk. On doubly banded trees more worms descended the branches from fruit remaining on the tree, being caught in the upper bands, than ascended from fallen fruit to be caught under the lower band.

Two years of bait trapping in Iowa orchards demonstrate the random distribution of populations from orchard to orchard. As previously suspected, however, brood flights were found to be generally earlier in the southern half of the state than in the north. The distribution of codling moth populations within an orchard as indicated by seasonal bait trap catches, demonstrates the heterogeneity of adult population from tree to tree.

Among the systematic variations of larval population may be mentioned location of fruit on a tree. From 24 trees in an orchard at Mitchellville, Iowa, all of which were treated alike, the average infestation in the tops was 18 per cent wormy and near the ground 14 per cent, a difference of four percentage units which was found to be statistically significant. Random selection of tree samples was therefore deemed important.

From 44 six-tree plots at Wenatchee, Washington, the worms per 100 apples were correlated with the percentage of wormy fruit, yielding a curved regression line fitted by the formula,

$$y = 1.049x^{1.1096}$$

with a standard error of estimate (worms per 100 apples from percentage wormy) of one worm per 100 apples for six tree means and 2.42 worms

[1] Original thesis submitted March, 1936. Doctoral thesis number 360.

per hundred apples for single trees. The correlation coefficient was 0.9092. Due to the low standard error of estimate and high correlation coefficient, counts of actual worms per 100 apples seemed unnecessary in most cases for the estimation of treatment effect.

The percentages of wormy fruit estimated with and without the inclusion of dropped fruit correlated very closely, the standard error of estimate (percentage wormy including drops from percentage wormy in picked fruit alone) being 3.45 per cent and the correlation coefficient, 0.987. The variety used in the tests was Ben Davis. In other localities and with other varieties these statistics would probably be different, but under the conditions of this experiment, the inclusion of the dropped fruits added little to the accuracy of the estimates.

A Latin square of four by four plots, each consisting of six trees, three of which were selected for harvest counts, was employed in the experimental orchard for testing differences in effect of spray treatments. From the analyses of variance and covariance it became obvious that no significant differences existed between the various plots of the orchard with respect to effect of location in the experimental block in this orchard. Neither were the differences between estimates of tree infestation based upon different sizes of samples larger than might normally be expected as long as estimates were made from purely random samples. A sample of 300 apples gave an adequate picture of tree infestation. The random variation between trees treated alike was very large and the analysis demonstrated beyond reasonable doubt that this was the major source of experimental error.

Crop size affects the infestation rather systematically and considerably, particularly between plot and test means. Small crops tend to be unusually wormy and those trees bearing large crops may be less heavily infested than normally expected. The worker must ascertain, then, whether a treatment applied to a certain set of plots is actually responsible for a low infestation or if the treatment has by chance been applied to plots bearing an unusually heavy crop which is the real cause of apparent good control. An analysis of covariance of the tests furnishes a fairly easy method of obtaining this information.

The four treatments involved were lead arsenate and lime, calcium arsenate used with a buffer of ferrous sulfate and lime, manganese arsenate plus fish oil as a sticker and a schedule employing three cover sprays of lead arsenate used against the first brood, followed by two cover sprays of summer oil and nicotine sulfate applied later in the season. The statistical analysis of the results from the tests used on the same trees in two orchards for the two seasons 1934 and 1935 indicate that the three arsenicals do not differ significantly in their effect on infestation; however, the schedule in which oil-nicotine is used against the second brood attack gives significantly better control of the codling moth. Due to the cost of this material, and the fact that it does not entirely solve the problem of excessive poisonous residue at harvest, its use can not be recommended without qualifications.

From the above outlined study of population variability it has been concluded that for the experimental conditions herein studied, a thoroughly efficient experimental analysis may be based on 300 apple samples selected at random from each tree and evaluated for percentage of

wormy fruit alone. The scoring of the dropped fruit and worms per 100 apples may be omitted without materially affecting differences due to treatment. The existence of a correlation between worminess and crop size, however, necessitates the inclusion of analysis of the covariance of these variables in reduction of data. Heterogeneity between trees within an orchard emphasizes the necessity of thorough replication.

# β-HYDROXYFURANS AND SOME OF THEIR BIOLOGICAL PROPERTIES[1]

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Although derivatives of hydroxyfurans are numerous and extremely important, there has been little investigation of the hydroxyfurans themselves. The availability of hydroxyfurans was considered, as well as a study of the chemical and biological properties of these compounds.

Since 3,4-dihydroxy-2,5-dicarbomethoxyfuran was more available than most of the described hydroxyfurans, this compound was used as the starting material for the preparation of the compounds investigated. Johnson and Johns[2] prepared 3,4-dihydroxy-2,5-dicarbomethoxyfuran by condensing diethyl oxalate with diethyl diglycollate in the presence of sodium ethoxide. These workers assigned the keto structure to the compound. Hinsberg[3] used the same reaction to prepare the dimethyl ester. It was found that the 3,4-dihydroxy-2,5-dicarbomethoxyfuran gave an intense violet color with ferric chloride solution. On the basis of the ferric chloride color test, Hinsberg favored the enol form of the compound. In the present investigation enol derivatives were obtained, but as yet no keto derivatives of 3,4-dihydroxy-2,5-dicarbomethoxyfuran were obtained. Among the enol derivatives of this type, which were prepared, were 3,4-diacetoxy-2,5-dicarbomethoxyfuran (m.p. 138°), 3,4-diacetoxy-2,5-dicarbethoxyfuran (b.p. 235°/35 mm.), the dicopper acetate salt of 3,4-dihydroxy-2,5-dicarbomethoxyfuran, 3,4-dibenzoxy-2,5-dicarbethoxyfuran (m.p. 146°), the diammonium salt of 3,4-dihydroxy-2,5-dicarbethoxyfuran (decomposition at 200°) and 3,4-dimethoxy-2,5-dicarbethoxyfuran (m.p. 48°).

Glyoxal and dimethyl diglycollate were condensed in the presence of sodium methoxide. The product of this condensation was acidic in behavior and on treatment with diazomethane, a small amount of substance melting at 104°-105° was obtained. This material, when mixed with dimethyl dehydromucate (m.p. 108°-109°) in 50-50 portions, melted at 106°-108°. This reaction gave support to the postulated furan structure for the condensation product of diethyl oxalate and dimethyl diglycollate in the presence of sodium methoxide.

The effect of the alkoxide was observed when diethyl oxalate and dibutyl diglycollate were condensed in the presence of sodium ethoxide. The condensation product was 3,4-dihydroxy-2,5-dicarbethoxyfuran (m.p. 186°).

Methylation of 3,4-dihydroxy-2,5-dicarbomethoxyfuran, using dimethyl sulfate in alkaline solution, resulted in two products. The main product was 3,4-dimethoxy-2,5-dicarbomethoxyfuran (m.p. 89.5°-90°),

[1] Original thesis submitted June, 1936. Original thesis number 385.

[2] Johnson and Johns, *Am. Chem. J.*, 36, 290 (1906).

[3] Hinsberg, *Ber.*, 45, 2413 (1913).

while about 10 per cent of total yield was 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran (m.p. 150°-151°). The separation of these two substances was accomplished by extracting the 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran with a saturated solution of secondary sodium phosphate. Several enol derivatives were prepared from the 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran, as well as from the other hydroxyfurans. Among them were 3-benzoxy-4-methoxy-2,5-dicarbomethoxyfuran (m.p. 117°-118°) and 3-acetoxy-4-methoxy-2,5-dimethoxyfuran (m.p. 108°).

Hydrolysis of 3,4-dimethoxy-2,5-dicarbomethoxyfuran in alkaline solution yielded 3,4-dimethoxy-2,5-dicarboxyfuran (m.p. 243°-245°; decompn.). No hydrolysis of 3,4-dihydroxy-2,5-dicarbomethoxyfuran in alkaline solution took place. A mono acid was obtained on alkaline hydrolysis of 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran. The methoxyl group seemed to influence the hydrolysis of the ester group when on the same side of the nucleus. The product of hydrolysis from 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran was postulated as 3-hydroxy-4-methoxy-2-carbomethoxy-5-carboxyfuran (m.p. 245°-246°). This compound gives a deep violet color with ferric chloride solution. The acid chloride of 3-hydroxy-4-methoxy-2-carbomethoxy-5-carboxyfuran melted at 132°-133.5°. A negative chloralide reaction resulted with 3-hydroxy-4-methoxy-2-carbomethoxy-5-carboxyfuran.

Decarboxylation of 3,4-dimethoxy-2,5-dicarboxyfuran gave 3,4-dimethoxyfuran (b.p. 172°-174° or 94°-96°/18 mm.; $D_{4^\circ}^{25^\circ}$ 1.1316; $N_D^{25^\circ}$ 1.4650). 3-Hydroxy-4-methoxy-2-carbomethoxyfuran (m.p. 100°-101°), obtained by a decarboxylation of 3-hydroxy-4-methoxy-2-carbomethoxy-5-carboxyfuran, was methylated to give 3,4-dimethoxy-2-carboxyfuran (m.p. 54°-55°), which on alkaline hydrolysis gave 3,4-dimethoxy-2-carboxyfuran (m.p. 170°-171°). When 3,4-dimethoxy-2-carboxyfuran was decarboxylated, 3,4-dimethoxyfuran (b.p. 172°) was obtained. These two preparations of 3,4-dimethoxyfuran gave identical maleic anhydride addition compounds (m.p. 92°-94°) as determined by a mixed melting point. The formation of a maleic anhydride derivative indicated the presence of a diene system which would be found if the furan nucleus was present in these compounds. 3,4-Dimethoxy-2-carbomethoxyfuran also forms an addition compound with maleic anhydride which melted at 109°-111°.

Treatment of 3,4-dimethoxyfuran with stannic chloride and acetic anhydride yielded two substances—a solid (m.p. 58°-59°) and a liquid. The liquid (b.p. 160°/8mm.; $N_D^{25^\circ}$ 1.5200) was oxidized to 3,4-dimethoxy-2,5-dicarboxyfuran. 3,4-Dimethoxy-2,5-diacetylfuran, prepared from the diacid chloride of 3,4-dimethoxy-2,5-dicarboxyfuran and dimethylcadmium, distilled at 160°/8mm. and had a refractive index of 1.5187 at 25°.

Oxidation of 3,4-dihydroxy-2,5-dicarbomethoxyfuran or 3,4-dimethoxy-2,5-dicarboxyfuran with dilute nitric acid yielded oxalic acid. An attempt was made to produce peroxides by passing oxygen through an ether solution of either 3-hydroxy-4-methoxy-2-carbomethoxyfuran or 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran, and in each case the result was negative. These results were not in accordance with those shown by 2,4,5-triphenyl-3-furanol[4], which formed the peroxide with ease.

[4] Kohler, Westheimer and Tishler, *J. Am. Chem. Soc.*, 58, 264 (1936).

Mercuration of 3,4-dimethoxyfuran demonstrated the extremely reactive nature of this type of compound. The only product isolated was 3,4-dimethoxy-2,5-dichloromercurifuran (m.p. 208°; decompn.). 3,4-Dimethoxy-2-carbomethoxyfuran yielded 3,4-dimethoxy-2-carbomethoxy-5-chloromercurifuran (m.p. 112°-113.5°) when treated with mercurating solution[5].

Bromination of 3-acetoxy-4-methoxy-2-carbomethoxyfuran yielded 3-hydroxy-4-methoxy-2-carbomethoxy-5-bromofuran (m.p. 125°-127°). This compound did not give a ferric chloride color test. When 3,4-dimethoxy-2-carbomethoxyfuran was treated with bromine in carbon tetrachloride, a compound which melted at 115°-116° was obtained. The compound was postulated as 3,4-dimethoxy-2-carbomethoxy-5-bromofuran.

Reduction of 3,4-dimethoxyfuran with Raney nickel at 2750 pounds of hydrogen at 200°-205° gave a liquid which distilled at 182°-183.5°. The refractive index of this liquid at 25° was 1.4378 and the specific gravity at 25° was 1.051. Erythrane[6] was methylated with dimethyl sulfate in alkaline solution and the product obtained distilled at 181°-183°. This liquid had a refractive index of 1.4395 at 25° and a specific gravity of 1.089 at 25°. The results from these two reactions were not satisfactory enough to claim complete identity of these two compounds.

In metabolism studies using *Aerobacter aerogenes* and zymin in a Warburg-Barcroft respirometer system 3-hydroxy-4-methoxy-2,5-dicarbomethoxyfuran showed the only significant action. This action of stimulation was comparable to that of the phenols. This compound showed no action as a growth stimulant, co-stimulant or hormone when tested in higher plants.[7]

[5] Gilman and Wright, *J. Am. Chem. Soc.*, 55, 3302 (1933).

[6] Henninger, Ann. chim. phys., (5), 7, 224 (1886).

[7] Dr. F. W. Went of California Institute of Technology carried out these tests.

# A STUDY OF THE GRAPHITIZATION OF IRON CARBIDE[1]

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In the heat treatment of steel the formation and decomposition of iron carbide plays a very important role. Probably for this reason investigations were first carried out to determine its stability.

Knowing the value for the change in free energy, $\Delta F$, of the reaction,

$$3\ Fe + C \rightarrow Fe_3C,$$

one can determine immediately whether iron carbide is stable or meta-stable. If the free energy of the compound is greater than the sum of the free energies of its elements, that is, if $\Delta F$ is greater than zero, the compound is meta-stable. If $\Delta F$ is less than zero, the compound is stable.

The following reactions were assumed to represent the mechanism for the graphitization and carburization of pure iron carbon compounds,

1. $Fe + 2CO \rightarrow Fe_3C + CO_2$
2. $C + CO_2 \rightarrow 2CO$
3. $3Fe + C \rightarrow Fe_3C.$

It is seen that reaction (3) is the sum of reactions ((1) and (2). If $\Delta F_1$, $\Delta F_2$ and $\Delta F_3$ represent the change in the free energy of reactions (1), (2) and (3), respectively, then,

$$\Delta F_3 = \Delta F_1 + \Delta F_2.$$

Both $\Delta F_1$ and $\Delta F_2$ can be determined experimentally, however, the equilibrium data for equation 2 in the International Critical Tables (1) were used in this investigation to obtain $\Delta F_2$.

$\Delta F_1$ was obtained by determining the equilibrium constant, K, for equation (1), and substituting in the equation,

$$\Delta F^\circ_1 = -RT\ln K.$$

Values for K were obtained at intervals of 25° from 550° to 900° C., by reading the concentrations of CO from a curve obtained by plotting percentage CO against temperature. The concentration of $CO_2$ was obtained by difference. The concentrations were changed to partial pressures and these values substituted into the equation,

$$K = \frac{P_{CO_2}}{P^2_{CO}}.$$

In order to determine the equilibrium pressures of CO and $CO_2$ in the presence of Fe and $Fe_3C$, an apparatus was developed so that the

[1] Original thesis submitted June, 1936. Doctoral thesis number 376.

gases were circulated continuously by means of an electromagnetic pump through the reaction chamber and the gas analysis apparatus. The reaction chamber was a furnace made of Armco iron. In the first part of the investigation Armco iron turnings were used; in the latter part, turnings from an Armco iron carbon alloy sample containing 1.55 per cent carbon were used. The gas analysis apparatus was a conductivity meter similar to those described by Daynes (2).

The change in free energy, $\Delta F^\circ_3$, for the reaction,

$$3Fe + C \rightarrow Fe_3C,$$

was obtained by adding $\Delta F^\circ_1$ and $\Delta F^\circ_2$.

The heat of formation of iron carbide was then calculated by substituting the values of $\Delta F_3$ into the equation,

$$\Delta H = \frac{d\frac{\Delta F}{T}}{d\frac{1}{T}}$$

and solving by a graphical method, i. e., by plotting the values of $\frac{\Delta F}{T}$ as ordinates and $\frac{1}{T}$ as abscissae. The slope of the curve so obtained gives $\Delta H$ directly.

The calculated values for the free energy change and the heat of reaction are recorded in Table 1.

The values for the heat of formation of iron carbide as obtained by various investigators, range from $-27{,}500$ to $+78{,}750$ calories. The values calculated from the experimental data secured in this investigation indicate a range of 5,450 to 40,700 calories over a temperature range of 550° to 850° C.

The values obtained for the free energy change of the reaction,

$$3Fe + C \rightarrow Fe_3C,$$

vary from $+5{,}943$ at 550° to $-4{,}948$ at 900° C. Values obtained by other investigators range from 11,598 calories to 2,281 calories, the temperature range being from 25° to 700° C.

The results obtained by using the free energy data in the International Critical Tables indicate that $Fe_3C$ is stable above 780° C. and metastable below this temperature.

TABLE 1. *Heat of formation of $Fe_3C$*

| Temp. °C. | $\Delta F_1$ | $\Delta F_2$ | $\Delta F_3$ | $\Delta F_4$ |
|---|---|---|---|---|
| 550 | — 298.3 | +6,241 | +5,493 | +34,200 |
| 575 | — 188.7 | +5,401 | +5,212 | +35,300 |
| 600 | — 149.5 | +4,318 | +4,168 | +38,000 |
| 625 | — 133.1 | +3,237 | +3,104 | +40,700 |
| 650 | — 51.2 | +2,105 | +2,054 | +34,600 |
| 675 | + 274.5 | +1,076 | +1,351 | +35,900 |
| 700 | + 846.1 | — 2 | + 844 | +14,700 |
| 725 | +1583.9 | —1,076 | + 508 | + 5,450 |
| 750 | +3017.5 | —2,150 | + 867 | |
| 775 | +3573.0 | —3,223 | + 350 | |
| 800 | +3811.1 | —4,471 | — 661 | +29,400 |
| 850 | +4030.6 | —6,543 | —2,512 | +32,600 |
| 900 | +4427.7 | —9,376 | —4,948 | |

By use of data taken from Garran (3), the results indicate $Fe_3C$ to be stable above 775° C. and meta-stable at temperatures below this value.

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# THE VALUE OF SEVERAL ORGANIC COMPOUNDS AS CONTACT AND STOMACH POISONS FOR CERTAIN INSECTS[1]

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## PART ONE

## THE OVICIDAL AND SCALICIDAL PROPERTIES OF SOLUTIONS OF DINITRO-O—CYCLOHEXYLPHENOL IN PETROLEUM OIL

The primary object of this part of the present investigation was to determine whether dinitro-o-cyclohexylphenol[2], which has shown rather unusual promise as a contact and stomach poison for certain insects, could be incorporated with a petroleum oil to obtain a more effective insecticide than the oil alone. Such a mixture of petroleum oil and dinitro-o-cyclohexylphenol would considerably reduce the amount of oil in the diluted spray, and in this way lessen the chances of tree injury without reducing the effectiveness of the mixture.

The toxicities of ammonium caseinate emulsions of dinitro-o-cyclohexylphenol dissolved in petroleum oil were determined in the laboratory for eggs of a plant bug, *Lygaeus kalmii* Stål, and for San José scale, *Aspidiotus perniciosus* Comstock.

A method was given for comparing the toxicities of substances to the Lygaeus eggs. In the method proposed, the embryonic and post-embryonic mortalities were pooled to furnish a measure of the total effectiveness of the ovicide.

A method was given for comparing the toxicities of contact insecticides to the San José scale and other scale insects. The general design can be employed for comparing the toxicity of substances particularly in cases where the viability of the insect population is significantly variable. The statistical methods employed are very useful for testing the adequacy of the experimental technique, the homogeneity of the populations, and for ascertaining whether the departures of the results from expectancy are of a magnitude ascribable to chance. The analysis demonstrated that conclusive results could be obtained with a heterogeneous insect population provided that homogeneous groups, each of sufficient size to provide for the desired treatments, could be drawn from the population.

Lethal concentrations for emulsions of dinitro-o-cyclohexylphenol dissolved in petroleum oil were established with respect to the amount of the dinitro-compound dissolved in the oil phase of the emulsions and the concentrations of oil plus the compound in the diluted sprays. The toxicities of the mixtures were represented by curves.

The results of the toxicity experiments demonstrated that only a relatively small concentration of oil was necessary to carry an effective

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[2] U. S. Patent No. 1,880,404.

concentration of the dinitro-compound. For example, a 100 per cent net mortality of San José scale was obtained with an oil mixture diluted to a spray strength of 1.0 per cent and containing 3.0 per cent of the dinitro-compound dissolved in the oil phase of the emulsion. Laboratory experiments have shown that a dilution of 3.0 per cent of petroleum oil without the dinitro-compound is necessary to furnish about an equally effective mortality of the scale. In the ovicidal experiments, a 100 per cent net mortality was obtained with a 1.0 per cent dilution of the oil mixture containing 6.67 per cent of the compound dissolved in the oil phase. A dilution of 3.0 per cent oil without the dinitro-compound gave only 59 per cent net mortality of eggs. These mixtures of petroleum oil plus dinitro-o-cyclohexylphenol considerably reduce the amount of oil in the diluted sprays without reducing the effectiveness of the mixture for control of the eggs and scale.

In view of the promising laboratory and field results, the use of the mixtures for control of insects during the dormant period can be recommended.

## PART TWO

## THE TOXICITY OF SOME NITRO-PHENOLS AS STOMACH POISONS FOR SEVERAL SPECIES OF INSECTS

Only a few compounds whose toxicities to certain insects have been evaluated on an individual dosage basis, have compared favorably in toxicity with the arsenicals. This part of the investigation includes the results obtained with a group of nitro-phenols of which 2-4 dinitro-6-cyclohexylphenol and some of its salts have shown considerable promise as stomach poisons for insects.

The following organic compounds were administered to the insects by a leaf-sandwich method: 2-4 dinitro-6-cyclohexylphenol and its calcium, magnesium, copper, and lead salts; calcium 2-6 dinitro-4-cyclohexylphenate; calcium 2-4 dinitro-6-phenylphenate; lead 3-5 dinitro-o-cresylate. Median lethal dosages (M.L.Ds.) were estimated for those compounds that were sufficiently toxic.

2-4 Dinitro-6-cyclohexylphenol and the calcium, magnesium, copper and lead salts were found to be several times more toxic than acid lead arsenate ($PbHAsO_4$) to the corn ear worm, *Heliothis obsoleta* Fab. Calcium 2-6 dinitro-4-cyclohexylphenate, calcium 2-4 dinitro-6-phenylphenate, and lead 3-5 dinitro-o-cresylate were not sufficiently toxic to estimate M.L.Ds. The indications were that deviations from the structure of the lethal phenol resulted in partial or complete loss of the high toxicity to insects.

Calcium 2-4 dinitro-6-cyclohexylphenate, which was the most toxic salt examined, was about 4.4 times more toxic than acid lead arsenate to the corn ear worm, 17 times more toxic than acid lead arsenate to the armyworm, *Cirphis unipuncta* Haw., and significantly more toxic than acid lead arsenate to the cabbage worm, *Ascia rapae* L.

The speed of toxic action for 2-4 dinitro-6-cyclohexylphenol and the four salts was several times greater than for acid lead arsenate. The mean survival times for the phenol and its salts ranged from 2 to 5 hours.

Arsenic trioxide ($As_2O_3$), 2-4 dinitro-6-cyclohexylphenol and the calcium salt were fed quantitatively in baits to the red-legged grasshopper, *Melanoplus femur-rubrum* DeGeer. The calcium salt displayed rather low toxicity, but the phenol was 2.5 times more toxic than arsenic trioxide. Furthermore, the speed of toxic action was approximately twice that of the arsenical.

The consistent and promising results obtained with 2-4 dinitro-6-cyclohexylphenol and the four salts, appear to recommend them for practical consideration as stomach poisons for mandibulate insects.

# THE PHYSIOLOGICAL ACTION OF SOME FURAN COMPOUNDS[1]

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A discussion of the physiological action of several furan compounds was made. Furan itself resembles benzene in its activity. Furfural and furfuryl alcohol are quite toxic to the animal organism. 2-Furoic acid is apparently without specific action. Other compounds mentioned were: the β-diethylaminoethyl esters of several furan acids which are mild local anesthetics; a series of mixed ketones containing the furyl radical, some of which are weak hypnotics; and a group of unrelated furan derivatives which have a very disagreeable action on man. This latter group of compounds may be classified as vesicants, lachrymators, and sternutators.

In order to clear up cases of questionable orientation in the furan nucleus a need arose for furantetracarboxylic acid. It seemed likely that the synthesis of the desired acid could be effected by the enolization and subsequent ring closure of dioxalsuccinic ester. Accordingly, the ester was prepared from sodio-oxalacetic ester, which on treatment with sulfuric acid gave tetracarbethoxyfuran which could be hydrolyzed with mineral acids to yield furantetracarboxylic acid. Through decarboxylation of this acid 3,4-furandicarboxylic acid and 3-furoic acid could be secured. It appears that this method of preparation of 3-furoic acid is a more convenient source than those previously used.

At present there is no unequivocal proof for the position of the nitro group in nitrofuran. 3,4-Furandicarboxylic acid is admirably suited to give conclusive proof as to whether the nitro group enters in the α- or β-position. With both β-positions blocked an entering group would have to assume an α-position. The resulting compound could then be decarboxylated and the residuum compared with the known mononitrofuran.

It appeared that 3,4-furandicarboxylic acid in the form of its ester should undergo nuclear substitution with extreme ease because there are two α-positions available and α-positions are very reactive. But a number of attempted nitrations were carried out in which the experimental conditions were made increasingly more drastic. Either no reaction occurred or the furan nucleus was ruptured by oxidation resulting in the formation of oxalic acid. In the case of halogenation, where more strenuous conditions may be employed without accompanying breakdown of the reactants, dimethyl 3,4-furandicarboxylate was found to undergo bromination in a sealed tube at 160° in the absence of a solvent to give a compound of undetermined structure.

It appeared that the oxidation of suitable dibenzofuran derivatives would furnish furantetracarboxylic acid. An investigation of the chemistry of dibenzofuran revealed that the matter of orientation in this heterocycle was in a vastly confused state, even for the most common simple

[1] Original thesis submitted December, 1935. Doctoral thesis number 354.

substitution products. The constitution of dibenzofuran is peculiar in that the nucleus possesses two distinct conflicting directive influences for nuclear substitution reactions. The position assumed by substituents in monosubstitution of dibenzofuran is apparently governed not only by the inherent characteristics of the molecule due to the diphenyl bond and the diphenyl ether linkage, and to experimental conditions, but also by the type of entering group. Since the diphenyl bond favors the 1- and 3-positions and the diphenyl ether linkage favors the 2- and 4-positions due to their strong *o*-, *p*-directing influence, it is not surprising that the substitution reactions of dibenzofuran are highly competitive and equally as uncertain.

The orientation of nuclear substitution reactions of dibenzofuran has been reviewed. Disubstitution reactions with unlike groups have not been investigated so extensively. The constitution of the nitration product of 3-acetaminodibenzofuran was confirmed and the product was 2-nitro-3-acetaminodibenzofuran. The compound secured after hydrolysis and replacement of the amino through diazotization was found to be 2-nitrodibenzofuran. And since the nitroacetamino compound was *ortho* substituted as was shown by the formation of quinoxaline derivatives from the reduced derivatives, there could be no doubt as to 2,3-disubstitution. In a similar manner the bromination product of 3-acetaminodibenzofuran was established as 2-bromo-3-acetaminodibenzofuran. The bromoacetamino compound was hydrolyzed and aminated in a sealed tube to give an *o*-diamine as shown by its ability to form quinoxaline derivatives. The bromoamine, secured by hydrolysis, on diazotization and replacement of the diazonium group by hydrogen yielded 2-bromodibenzofuran.

A series of 3-N-alkylated aminodibenzofurans was prepared for pharmacological testing. The following compounds were made: dimethylamino-, diethylamino-, methylamino-, ethylamino-, propylamino-, and N-piperidinodibenzofuran. The Skraup reaction on 2- and 3-aminodibenzofurans produced two isomeric pyrido-derivatives in each instance. These nitrogen heterocycles as well as some of their reduced derivatives were tested for physiological action.

Other amino derivatives whose physical properties have been described elsewhere were synthesized and tested. 2-ω-Diethylaminoacetyldibenzofuran and 2-ω-piperidinoacetyldibenzofuran were secured on treating 2-chloroacetyldibenzofuran with diethylamine and piperidine. From these tertiary amines on reduction with Adams catalyst there was obtained diethylaminomethyl-2-dibenzofurylcarbinol and piperidinomethyl-2-dibenzofurylcarbinol. A reaction was effected between 2-dibenzofurylmagnesium bromide and α, β-dichlorethyl ether to give the ethel ether of chloromethyl-2-dibenzofurylcarbinol. Upon treatment of this ethoxy compound with piperidine there resulted the ethyl ether of piperidinomethyl-2-dibenzofurylcarbinol. Chloromethyl-2-dibenzofurylmethylcarbinol, prepared from the Grignard reagent of 2-bromodibenzofuran and epichlorohydrin, furnished diethylaminomethyl-2-dibenzofurylmethylcarbinol on reacting with diethylamine.

Several compounds were prepared which have not previously been reported. A Gabriel phthalimide synthesis on 4-β-bromoethyl- and 2-β-bromoethyldibenzofuran yielded 4-β-aminoethyldibenzofuran, distilling at 165°-166°/2mm., and 2-β-aminoethyldibenzofuran, b.p. 167°-170°/2mm.

The hydrochlorides of these amines melted at 263° and 278°, respectively. From the 4-isomer there was obtained, on treatment with methylal and acid, tetrahydropyrido-[5,4-c]-dibenzofuran, which distilled at 183°-184°/1-2mm. and whose hydrochloride melted at 259°.

2-Chloromethyldibenzofuran was prepared by saturating a petroleum ether (b.p. 75°-115°) solution of dibenzofuran with hydrogen chloride in the presence of zinc chloride and trioxymethylene, and melted at 78.5°-79.5°. This halogen compound was converted to 2-cyanomethyldibenzofuran in the customary manner, and was found to melt at 102.5°-103.5°. Reduction of the nitrile with Adams catalyst yielded 2-$\beta$-aminoethyldibenzofuran.

The pharmacological reports revealed that the dibenzofuran derivatives investigated in this study are relatively toxic and that they possess physiological action of meager therapeutic importance. It is, however, significant that the 4-aminodibenzofuran and 4-acetaminodibenzofuran show analgesic action.

# A STUDY OF SOME LIPOLYTIC MICROORGANISMS ISOLATED FROM DAIRY PRODUCTS[1]

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The ability of an organism to hydrolyze fat appears to be an important character and in the future will be considered along with the other characters in preparing descriptions of species. The need for simple and reliable methods of determining lipolysis is therefore apparent. The objects of the investigation were to consider some of the methods used for the detection of bacterial lipolysis and to isolate and study a number of lipolytic microorganisms commonly found in dairy products.

## SECTION I. OBSERVATIONS ON THE METHODS USED FOR THE DETECTION OF BACTERIAL LIPOLYSIS

The nile blue sulfate technic consisted of the addition of 0.5 ml. of a fat emulsion (2 per cent cottonseed oil in 0.5 per cent agar) to each plate before pouring with beef infusion agar containing nile blue sulfate in the proportion of 1 part dye to 10,000 parts of the agar; lipolysis was detected by a change in color of the globules in the vicinity of lipolytic colonies from pink to blue. Due to the inhibition of many non-lipolytic organisms, this technic was found useful for the isolation of lipolytic types when they were present in small numbers as compared with the total numbers. However, a disadvantage of the method was that certain lipolytic types were also inhibited.

The modified nile blue sulfate technic was carried out by growing the organisms on plates containing dispersed cottonseed oil or butter fat and, after incubation, flooding the plates for 30 minutes with a 1 to 1,500 aqueous solution of the dye. Globules in the vicinity of lipolytic colonies were stained blue while those at a distance were stained pink. The modified method was very valuable when total and lipolytic counts were desired. However, when the proportion of lipolytic organisms to total organisms was low the modified method was not especially useful.

The simple triglyceride technic consisted of dispersing tripropionin or tributyrin in agar used for growing organisms and detecting lipolysis by a disappearance of the globules. The simple triglyceride technic was not an accurate method for determining lipolysis since some cultures of *Streptococcus lactis* were found to hydrolyze tripropionin and tributyrin but not cottonseed oil or butter fat.

In the natural fat technic emulsified cottonseed oil was added to agar used for growing the organisms and lipolysis was detected by a change in the appearance of the fat globules. The technic was well adapted to the study of lipolysis because relatively high total and lipolytic counts could be obtained and the picking of lipolytic colonies was not complicated by

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flooding the plates. A disadvantage of the technic was the impossibility of detecting lipolytic bacteria when they were present in small numbers as compared to the total numbers.

## SECTION II. DISTRIBUTION OF LIPOLYTIC MICROORGANISMS IN DAIRY PRODUCTS

Lipolytic bacteria were found to be distributed widely in both normal and abnormal samples of milk, cream and butter. Many of the samples of these products, especially if they were held at comparatively low temperatures for extended periods, yielded cultures of *Pseudomonas fragi.*

Attempts to isolate lipolytic microorganisms from milk obtained from the vat immediately after pasteurization invariably resulted in failure although they had been shown to be present before heating. This suggests that the presence of lipolytic organisms in pasteurized dairy products is due to contamination after heating.

In addition to the materials already mentioned the plating of miscellaneous dairy products and various other materials yielded numerous cultures of lipolytic microorganisms.

## SECTON III. THE IDENTIFICATION AND CLASSIFICATION OF CERTAIN OF THE ORGANISMS ISOLATED

A large number of lipolytic cultures were isolated during the plating of various dairy products and miscellaneous materials.

Most of the lipolytic cultures obtained were apparently *Ps. fragi.* Fifty-eight of the cultures thought to be *Ps. fragi* were studied in detail and found to agree with the organism described by Hussong (3). *Ps. fragi* was found to be widely disseminated and was one of the organisms encountered most frequently in the spoilage of certain dairy products. The defect produced was either a typical rancidity or an odor suggesting *Ps. fragi.*

A new lipolytic species, *Achromobacter oleifindens,* was isolated and described. *Ach. oleifindens* differed from the more common lipolytic species because of the acid coagulation of litmus milk and of the failure to digest milk. The organism was not proteolytic and did not produce rancidity in butter.

A number of inert lipolytic cultures were obtained and were considered identical with *Bacillus abortus* var. *lipolyticus* described by Evans (1). A study of the morphology, cultural characters, and biochemical features of the organism permitted an extension of the description given by Evans. The characters of the species indicated it belonged in the genus Alcaligenes and the name *Alcaligenes lipolyticus* was proposed. The organism produced rancidity in butter and was characterized by its ability to rapidly hydrolyze fat and to use salts of certain of the fatty acids as the sole source of carbon.

A number of cultures of a yeast that was lipolytic, as well as proteolytic, were studied and found to be *Mycotorula lipolytica,* which was investigated by Harrison (2). The cells of this organism were ellipsoidal to cylindrical with occasional hyphal-like threads. The yeast attacked fat readily and some cultures produced rancidity in butter while others produced cheesiness. The organism grew readily on ordinary laboratory media.

The results obtained on the cultures studied indicated that even such a character as the ability to hydrolyze fat may not be stable. This was especially true with certain of the cultures of *Ps. fragi*. The failure of some of the lipolytic cultures studied to produce rancidity in butter was thought to be due to poor growth or no growth in butter; in some cases when an organism was proteolytic as well as lipolytic a cheesy condition rather than rancidity resulted.

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# FEASIBILITY OF CERAMIC PRODUCTS AS TRICKLING FILTER MEDIA[1]

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Trickling filters are beds of rock or other materials on to which sewage or other waste is sprinkled or otherwise distributed and through which it trickles or percolates in the process of purification. Purification is brought about largely as a result of the biological life which is present in the jelly-like slime or film which soon develops on each individual piece of filter medium.

It has been held that the operation of the filter is dependent upon the effective surface of this microbial film, and the ease with which the oxygen of the air can reach all parts of this film. If this is true then specially preformed material having especially large interstitial spaces and a maximum amount of surface per unit of volume will have marked advantages. Ceramic products such as absorption tower packing offer a maximum surface as well as a maximum interstitial space. With this in mind the feasibility of using ceramic products as trickling filter media was investigated.

In a preliminary experiment two filters, each four square feet in area and six feet in depth, were dosed intermittantly with a synthetic waste. One to three-inch granite and one-inch Raschig rings were used as the media in the two filters. The rate of application was maintained constant at 2 M.G.A.D. with a six minute dosing cycle. A synthetic waste was produced continuously from dried sheep manure and later from a mixture of dried sheep manure and spray dried skim milk powder. On the basis of concentration of applied waste the operation of this experimental plant may be divided into three periods. During the first period from May 23 to June 20, 1933, the average B.O.D. concentration of the applied waste was 117 p.p.m. and the effluents from the Raschig ring and granite filters contained 18 and 21 p.p.m., respectively. During the second period from June 20 until August 25, the average concentration of the applied waste was 567 p.p.m. and the effluents were 46 and 58 p.p.m. for the Raschig rings and granite, respectively. During the third operating period from August 25 until September 28, the average concentration of the applied waste was 999 p.p.m., although at times the concentration was as high as 1400 p.p.m. The concentrations of the effluents obtained were 146 and 309 p.p.m. for the Raschig rings and granite, respectively.

In spite of the high concentration and rate of application of the waste the filters at no time clogged to the extent of making remedial measures or stoppage of the filters necessary, although the granite did, at times, pond.

In this preliminary experiment the Raschig ring filter showed better B.O.D. reduction, more uniform operation and greater nitrification than

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the granite control filter. Dried sheep manure was not found satisfactory for the production of a uniform concentration of "synthetic" waste.

Seven different filter media were placed into filters, each of four square feet area and six foot depth. The filter media used were one to three-inch granite, ¾, 1, 1½, and 2¼-inch Raschig rings, a special block designated as Straight's block and corn cobs. The ceramic media used in this and in the preliminary investigation were produced from Iowa clays. These experimental filters were placed into operation at the sewage disposal plant of the City of Ames, and were dosed with settled sewage from the effluent from the settling chamber of the city Imhoff tank. The filters were dosed at a very constant rate by means of a motor operated dosing device. Chemical analyses were made on 24-hour and bi-weekly chloroformed composite samples of both the influent and the effluents.

The operation of this experimental plant may be divided into three operating periods on the basis of the rate of application of the sewage. During the first operating period from August 16 until December 9, 1934, the rate of application was 2 M.G.A.D. with a six minute dosing cycle. During the second operating period from December 9, 1934, until April 1, 1935, the rate of application was 4 M.G.A. D. with a 3 minute dosing cycle. During the third operating period from April 1 until June 18, the rate of application was 8 M.G.A.D. with a 3 minute dosing cycle. The experimental plant has been continued in operation since the third operating period at a rate of 16 M.G.A.D. with a 3 minute dosing cycle.

The average B.O.D. concentration of the influents during each of the three operating periods was about 190 p.p.m. At no time during the operation of the plant did clogging or ponding occur. During the first two operating periods the effluent rfom the three-fourths-inch ring contained the least B.O.D. with the larger sizes of rings containing progressively greater amounts. During these two periods of operation the B.O.D. of the effluent appeared to be a function of the surface of the filter media. During the third operating period the effluents from all the filters contained nearly the same concentration of B.O.D., with a slight advantage in favor of the larger rings. It was postulated that during this period the ventilation obtained in the filters became the limiting factor in B.O.D. removal and that as a result the larger ring filters, since they offered less resistance to natural ventilation, were able to produce an effluent of lower B.O.D. concentration.

An unusual amount of nitrfication was obtained in the three-fourths-inch ring filter. Average nitrate concentration of 18.6, 17.0, and 14.0 p.p.m. as nitrogen were obtained during the three operating periods from this filter. Progressively smaller amounts of nitrates were obtained from the larger sizes of rings. It was found that throughout the operation of the filters the nitrification obtained appeared to be a function of the surface of the media.

There was some evidence presented to show that the limiting factors for nitrification and B.O.D. removal are not the same.

Studies were made of the rates of runoff from the filters for various rates of application and various dosing cycles, both before and after the microbial film had developed. It was found that the microbial film had a very marked effect in smoothing out the flow through the filter. It was found that the flow of water through the filters was markedly different

than that of sewage. It was shown that this difference was in part caused by the difference in surface tension of water and sewage.

The least variation in runoff rates occurred in the filters having the smallest medium. That is, the larger surface of the medium serves to delay or impede the flow through the filter and to smooth out the flow.

After the microbial film had developed the variation in rate of runoff from the three-fourths-inch ring filter was found to be very small. Inspection of the curves obtained after the film had developed indicates that dosing cycles of less than 3 minutes are desirable, especially at the higher rates of waste application.

An estimate is presented for the cost of manufacture of Raschig rings. It appears probable that one-inch Raschig rings can be produced under competitive conditions for less than $5.00 per cubic yard. Further, it appears probable that Raschig rings can, because of the greater capactiy and greater efficiency obtained, successfully compete with the materials now commonly used for trickling filter media.

# THE RELATIVE REACTIVITIES OF SOME ORGANOMETALLIC COMPOUNDS[1]

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In organic synthesis the use of selective or preferential reactions with compounds having polyfunctional groups is of very great importance. The determination of the relative rates of reaction of members of groups or classes of compounds with certain functional groups affords a method for estimating the proper reagents to use in reactions where more than one functional group is involved. In this study an attempt has been made to compare the relative reactivities of the organometallic compounds of aluminum, boron and zinc with several selected reactants. It was hoped that by the use of these less reactive organometallic compounds new syntheses could be realized with polyfunctional compounds not otherwise attainable with the more reactive organometallic compounds.

The color test for reactive organometallic compounds was used in these rate studies. The color test, as originally developed, was used only with the more reactive organometallic compounds formed from metals in the first and second groups of the periodic table. Positive color tests were obtained by the reaction of the organometallic compounds of aluminum, boron and zinc on Michler's ketone provided fairly concentrated solutions, higher temperatures and longer periods of contact were used. In order to obtain a good color test with these less reactive organometallic compounds it was found necessary to use solutions of approximately 1 molar concentration. More dilute solutions were either heated at 100-110° for 15 minutes, as in the case of the less reactive organozinc compounds, or allowed to stand with Michler's ketone for as long as 48 to 60 hours. This suggested the possibility that other reactions which do not go under ordinary conditions may proceed very slowly and require considerable time for completion. In this connection several organometallic compounds of Hg, Pb, Sn and Bi were sealed in test tubes with a 1 per cent Michler's ketone solution to determine if after prolonged contact reaction had taken place.

A survey of the literature shows that practically the only reactions of organo-aluminum compounds reported were condensation reactions. In most cases reported concentrated solutions of the mixed organo-aluminum compounds were reacted with different functional groups. In this work more dilute solutions of the simple organo-aluminum compounds were studied. Several reactions of tri-p-tolylaluminum, triphenylaluminum and tri-*n*-propylaluminum have been investigated and found to proceed in the normal manner of the more reactive organometallic compounds but at a slower rate. Thus, triphenylaluminum gave 23.1 per cent yields of triphenylcarbinol after standing 16 days at room temperature with benzophenone. Similar reactions were carried out between organo-aluminum

[1] Original thesis submitted August, 1935. Doctoral thesis number 343.

compounds and phenyl isocyanate, benzaldehyde, carbon dioxide and benzonitrile. The use of xylene solutions for the preparation and reactions of organo-aluminum compounds was found to be very advantageous.

The reactions of organoboron compounds were also investigated. No reactions wtih functional groups have hitherto been reported, and the only reactions entered into by these compounds were oxidation, hydrolysis, ammination and the addition of alkali metals. The reaction of triphenylboron with phenyl isocyanate and benzaldehyde was investigated and the expected products were isolated in 16.2 per cent and 10 per cent yields, respectively. The products were not isolated from the reaction of triphenylboron with benzophenone or benzontrile. It was not possible to isolate the expected products from the reaction of tri-*n*-propylboron with various functional groups. Several difficulties were experienced in working with organoboron compounds and the field contains many interesting problems. The preparation and purification of these organoboron compounds has been described.

The reactions of organozinc compounds are very numerous, but, in general, these compounds are considered as not reacting with the carbonyl linkage. Diphenylzinc has been found to react with carbon dioxide, benzophenone, phenyl isocyanate and benzontrile. The reaction of di-*n*-propylzinc with phenyl isocyanate was the only reaction tried using the alkyl compounds, and since the normal product was obtained here further studies were not made.

To determine the relative reactivities of these different organometallic compounds 50 cc. of a 0.2 molar solution of these compounds in xylene was treated with a 10 per cent excess of the reactant. Two cc. portions were removed at regular intervals and tested for the presence of organometallic compounds. The time required for the completion of the reaction was determined in this manner for each organometallic compound. In this manner the reaction rates of the phenyl derivatives of aluminum, boron and zinc were compared with one another and with the *n*-propyl and p-tolyl derivatives of the same metals. Benzaldehyde, benzophenone and benzonitrile were the reactants used. In general, the organo-aluminum compounds were most reactive, the organoboron compounds were next, and the organozinc compounds least reactive with the reactants studied. The *n*-propyl and p-tolyl derivatives appeared to be more reactive than the phenyl derivatives. The relative order of decreasing reactivities of the various functional groups studied seemed to be the same as previously reported; that is, benzaldehyde, benzophenone and benzonitrile.

The minimum concentration of the phenyl and *n*-propyl derivatives of aluminum, boron and zinc which were required to give a positive color test was determined and the relative rates of reaction of dilute solutions of these derivatives with Michler's ketone were observed. In each case the order of decreasing reactivity seemed to be: organo-aluminum compounds, organoboron compounds, organozinc compounds.

The preparation of organo-aluminum and -zinc compounds by the displacement of mercury from organomercury compounds by aluminum and zinc, respectively, affords a method of comparing these two organometallic compounds. By use of a qualitative test for organomercury compounds the length of time required for the disappearance of organomercury compounds in these solutions was determined. It was found that in a

boiling xylene solution aluminum would replace mercury in approximately 2½ hours, while zinc required 6 to 8 hours.

Some rather definite correlations have been made between the relative reactivities of the organometallic compounds and the position of the metal in the periodic table and in the electrochemical series.

## SUMMARY

1. The color test for reactive organometallic compounds has been applied to the lesser reactive organometallic compounds.

2. The preparation and reactions of organometallic compounds of aluminum, boron and zinc have been studied.

3. The relative rates of reaction of the *n*-propyl and phenyl derivatives of aluminum, boron and zinc with Michler's ketone, benzaldehyde, benzophenone, and benzonitrile have been studied.

4. The order of decreasing reactivities has been found to be: organoaluminum compounds, organoboron compounds, organozinc compounds.

5. A correlation has been made between the relative reactivities of some organometallic compounds and the position of the metals in the periodic table and in the electrochemical series.

6. The probability of the preparation of otherwise inaccessible compounds through the medium of selective or preferential reactions involving these less reactive organometallic compounds has been discussed.

# THE DECOMPOSITION OF SOME HUMUS-FORMING MATERIALS IN SOILS[1]

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Experiments were conducted to study the influence of oat straw, wheat straw, sudan grass, cane sorghum, flax, cornstalks, millet, hemp, soy beans, alfalfa, sweet clover, and red clover on the numbers of cellulose decomposing organisms in the soil. It was observed that each material brought about an increase in the number of cellulose decomposing organisms but there was no consistent difference in number of organisms in the soils treated with the different plant materials.

The respiration chamber method was used for measuring carbon dioxide production in soils. A film was found to form over the surface of the barium hydroxide in the presence of carbon dioxide. Preliminary studies showed that agitating the barium hydroxide in the bottom of the chamber did not give any increase in the amount of carbon dioxide absorbed. It was concluded, therefore, that all of the carbon dioxide was absorbed when the chambers were stationary. An analysis of the atmosphere within the chamber during the progress of an experiment indicated that the oxygen content was normal and that there was no accumulaion of carbon dioxide in the air of the chamber. The respiration chamber method for measuring carbon dioxide production in soils was preferred to the aspiration method as the respiration chamber method required less equipment and less time for the determinations than the aspiration method.

The evolution of carbon dioxide from the soil treated with the different plant materials was measured by the chamber method during 190 days. The results obtained showed that the materials containing the most nitrogen produced the most carbon dioxide during the first few days of the experiment, but after this time the materials containing small amounts of nitrogen produced the most carbon dioxide. The various plant materials listed in descending order of the amount of carbon dioxide produced after 228 hours are sweet clover, alfalfa, soy beans, flax, red clover, millet, hemp, sudan grass, cane sorghum, cornstalks, oat straw and wheat straw. Plotting the logarithm of the mean carbon dioxide evolved from the soils treated with non-legumes against the logarithm of time from 15 days until the end of the experiment gave a straight line. The slope of the line for the non-legumes was greater than the slope of the line for the legumes.

Soils treated with the plant materials at the rate of 0.3 per cent and 4.0 per cent showed a nitrate depression directly related to the nitrogen content of the plant materials added. An r value of 0.95 was obtained with the 0.3 per cent treatments and an r value of 0.99 was obtained with the 4.0 per cent treatments. This indicates that under favorable soil conditions the rate of organic matter decomposition is significantly correlated with the nitrogen content of the organic matter.

[1] Original thesis submitted June, 1936. Doctoral thesis number 363.

The nitrogen content of these plant materials varied between wide limits, but the carbon contents varied little. The different plant materials were found to differ greatly in base exchange capacity. The base exchange capacity of the various materials was correlated with the nitrogen and lignin content, but not with the alcohol benzene fraction or the hydrolyzable fraction. The base exchange capacity of the different plant materials increased as the materials decomposed. The increase in exchange capacity was found to be significantly correlated with the increase in lignin content. Many of the plant materials did not differ greatly in exchange capacity after decomposition but, in general, as decomposition proceeded the leguminous plant materials continued to possess a higher base exchange capacity than the non-leguminous plant materials. The difference became less as decomposition proceeded. As the plant materials decomposed the exchange capacity increased much more rapidly than the lignin content or the decrease in weight. At the end of 210 days there was no definite relation between the exchange capacity and the lignin content, the alcohol-benene-soluble fraction or the amount of hydrolyzable material.

Base exchange studies were made on soils treated with the different plant materials at the rate of 5.0 per cent, and which had been allowed to decompose for 289 days under laboratory conditions. The results showed that the base exchange capacity of the soils was significantly correlated with the nitrogen content of the plant materials added. The soils receiving plant materials of high ntirogen content were highest in the base exchange capacity.

The base exchange capacity of soils treated with 4 per cent of the different organic materials was determined at intervals as the materials decomposed. The results obtained showed an increase in exchange capacity in each case. Some of the plant materials increased the exchange capacity of the soil more than others. Three plant materials apparently reached a maximum in base exchange capacity before the last sampling as the exchange capacity was lower than at the previous sampling.

Analyses of the undecomposed and decomposed plant materials showed that the lignin content increased as decomposition proceeded. However, the lignin content of the decomposed plant materials calculated on the basis of the original organic matter showed a significant decrease in the lignin content. The losses in lignin were found to range from 24.71 per cent with cane sorghum to 54.80 per cent with soy beans.

# THE ELECTRON-SHARING ABILITY OF ORGANIC RADICALS. THE TERPENES AND RELATED COMPOUNDS

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The modern electronic theory of valence seems to offer the most adequate explanation of the properties of organic compounds available at the present time. According to this theory the properties of a molecule are dependent upon its electronic configuration and these properties may be varied by shifting this electronic configuration. The introduction of a substituent into an organic molecule, therefore, causes a change in the properties of the molecule because of a difference in the ability of the substituent to share electrons with, or transfer them to or from, the atoms to which they are linked. One of the most readily determined properties of the organic acids and amines is their apparent degree of ionization.

Ostwald was the first to observe that the ionization constant of organic acids changed by a definite amount when a substituent was placed in the same position relative to the carboxyl group. Wegscheider extended the investigations of Ostwald and summarized the available data into tables of factors which represented the effect of the position of various substituents upon the dissociation constants of organic acids. This "Ostwald Law" has been the basis for a great deal of experimental work.

Derick attempted to establish a standard for determining the effect of introducing a radical into a molecule. He attempted to use the acidic and basic dissociation constants of certain hydroxides. Because of the apparent lack of ionization in so many compounds this standard was difficult to apply experimentally.

Hixon and Johns and co-workers have demonstrated a mathematical relationship which places organic radicals attached to a polar group in a definite order in a series. This relationship for the acids and amines was of the type:

$$\text{Log } K = ke^{ax+b} + C$$

where log K is the logarithm of the dissociation constant and x is the abscissa value, or "electron-sharing ability," of the radical. The radicals considered must contain no polar group themselves. It was further pointed out that the electron-sharing ability appeared to be a function of the mass of the radical and the spatial configuration of the molecule as well as the potential of the atoms.

The investigation presented here was undertaken to determine the ionization constants of some terpene amines and to predict, if possible, some types of structure the amines of which might have dissociation constants in the range $10^{-5}$-$10^{-9}$. Qualitative observations were made on the degree of stability of the amines studied.

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The hydrochlorides of the following amines were prepared and purified:

(1) Bornyl amine
(2) Pinyl amine
(3) α-Aminoamyl methyl ketone
(4) Camphor amine

1-Menthyl amine was obtained from Eastman Kodak Company and its hydrochloride purified.

The ionization constants of the amines just mentioned were measured by a modification of the half neutralization method. The data obtained are tabulated in the accompanying table.

*Tabulated Results from E.M.F. of Cells of Half Neutralized Amines*

$Pd/H_2$(1 atm.) amine sol.//KCl(sat.), $Hg_2Cl_2/Hg$

| Substance | Wt .of Amine. HCl | cc.'s NaOH 0.01586N | Pressure mm. Hg | E.M.F. Obs. | E.M.F. 760 mm. | -log $K_B$ |
|---|---|---|---|---|---|---|
| 1-Menthyl amine | 0.0859[1] | 14.12 | 738 | 0.8478 | 0.8486 | 3.81 |
| | 0.1023[1] | 16.85 | 738 | 0.8489 | 0.8497 | 3.79 |
| Bornyl amine | 0.1025[2] | 17.04 | 734 | 0.8290 | 0.8299 | 4.17 |
| | 0.0410[1] | 6.82 | 742 | 0.8294 | 0.8302 | 4.12 |
| Pinyl amine | 0.0837[1] | 14.06 | 734 | 0.7522 | 0.7531 | 5.42 |
| | 0.1002[1] | 16.84 | 734 | 0.7521 | 0.7530 | 5.42 |
| α-Aminoamyl methyl ketone | 0.0487[3] | 9.27 | 737 | 0.7287 | 0.7296 | 5.82 |
| | 0.0609[3] | 11.55 | 731 | 0.7300 | 0.7310 | 5.80 |
| | 0.0543[3] | 10.34 | 726 | 0.7302 | 0.7313 | 5.79 |
| Camphor amine | 0.2065[3] | 18.13[4] | 742 | 0.6644 | 0.6652 | 6.91 |
| | 0.2052[3] | 18.02[4] | 742 | 0.6697 | 0.6705 | 6.82 |
| | 0.2036[3] | 17.88[4] | 742 | 0.6644 | 0.6652 | 6.91 |
| | 0.2046[3] | 17.96[4] | 742 | 0.6611 | 0.6619 | 6.96 |

[1] Made up to 100 cc. solution.
[2] Made up to 250 cc. solution
[3] Made up to 50 cc. solution.
[4] Normalcy 0.02797.

Some observations upon the structures of the amines studied in this investigation might prove of interest. Camphor amine, with an ionization constant of $1 \times 10^{-7}$, was the most negative. Bornyl amine, with a similar structure, proved to be much more positive ($K = 7 \times 10^{-5}$). Even though the amino group is on the 4-position in the first case and on the 3-position in the second, the only major difference between the two compounds is the presence of a carbonyl group in camphor amine. The larger part of the difference in the ionization constants of these two compounds must be due to the negativity of this grouping. This contention is further strengthened by the constant obtained for α-aminoamyl methyl ketone

($1.6 \times 10^{-6}$). All previous data obtained in this laboratory indicate that, if the oxygen in the above compound be replaced by two hydrogen atoms, the magnitude of the ionization constant should increase to about $10^{-4}$. The ring structure of camphor probably accounts for the lower ionization constant of its amine. The effect of the bridge-ring is also observable in the cases of menthyl and bornyl amines. Pinyl amine illustrates the effect of the four membered bridge-ring as contrasted with the five membered ring of the bornyl radical. The methylene group one carbon removed from the amino radical without doubt contributed to the negativity of the pinyl structure.

Some bicyclic terpene amines containing a three membered ring may possibly prove still more negative.

## CONCLUSIONS

From the data presented in this study the following conclusions may be drawn:

1. The terpene amines have ionization constants ranging from about $10^{-4}$ to $10^{-7}$.

2. There is evidence of instability in those amines with constants of the order of $10^{-6}$ to $10^{-7}$.

3. There is qualitative evidence indicating the existence of a minimum stability range in the electron-sharing ability curve for amines.

4. The instability of primary amines of the terpene type explains the lack of values in the literature for ionization constants ranging from $10^{-5}$ to $10^{-9}$.

5. So-called aliphatic amines may be modified by placing a carboxyl group in the alpha position, to give ionization constants and stabilities within this unstable range.

6. Ionization constants may be determined by a modification of the half neutralization method, using the amine hydrochloride and a half-equivalent of NaOH instead of the usual procedure.

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# PHYSIOLOGY OF THE LACTIC ACID BACTERIA[1]

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The true lactic acid bacteria constitute a large and ubiquitous group of microorganisms forming large quantities of lactic acid from carbohydrates. The group is conveniently subdivided into homofermentative and heterofermentative bacteria. The former produce substantially nothing but lactic acid from glucose with essentially traces of carbon dioxide, acetic acid and glycerol, whereas the hetero-forms yield relatively large quantitis of ethyl alcohol, carbon dioxide, acetic acid and glycerol in addition to lactic acid.

Relatively few investigations have dealt with the dissimilation of carbohydrates by the lactic bacteria, in particular in the case of the hetero-lactic forms.

A careful study of the dissimilation of carbohydrates brought about by the lactic bacteria should prove of value to agriculture, the fermentation industry and to systematic bacteriology.

The present investigation involves primarily a study of the mechanism of the dissimilation of glucose and levulose by identified and well known cultures as follows: *Lactobacillus lycopersici, L. mannitopoeus, L. plantarum, L. acidophilaerogenes, L. cucumeris, L. gracilis, L. fructivorans* and *Leuconostoc dextranicus.*

Quantitative studies of the dissimilation of glucose and levulose were made; intermediary products were determined by the addition of fixatives and the breakdown of the intermediaries was studied.

The quantitative relationships established among the final products lead to certain conclusions as to the mechanism of dissimilation.

The fermentation of glucose by both homo and hetero bacteria yielded acetic acid and carbon dioxide in equimolar quantities. It is probable that these two compounds originated from the breakdown of a single 3-carbon intermediary. The formation of acetic acid and carbon dioxide from the secondary fermentation of lactic acid by hetero lactic forms is suggested by the results. Pyruvic acid would be a precursor of the 2- and 1-carbon compounds according to the Embden-Meyerhof scheme of muscle and yeast dissimilation. This compound was fermented to acetic acid, carbon dioxide and lactic acid. However, pyruvic acid was isolated as an intermediary in the aerobic breakdown of lactic acid. The reaction between pyruvic acid and lactic acids may prove to be reversible.

The formation of carbon dioxide and acetic acid is an oxidative change and must be accompanied by a reduction product. This requirement was met by the formation of glycerol equal to twice the acetic acid.

Ethyl alcohol, formed by the hetero-group, was accompanied by equimolar quantities of carbon dioxide, indicating the formation of the alcohol by the breakdown of a 3-carbon intermediary. Evidence of this assump-

[1] Original thesis submitted March, 1936. Doctoral thesis number 358.

tion was shown by serial analyses of glucose fermentations. The percentage of glucose fermented to lactic acid decreased while that fermented to acetic acid, carbon dioxide and ethyl alcohol increased. The latter three compounds are thus formed at the expense of the lactic acid either by the breakdown of lactic acid itself or by an increase in the rate of their formation from a precursor of lactic acid. The formation of ethyl alcohol by the decarboxylation of lactic acid has never been shown, to the knowledge of the author. According to the Embden-Meyerhof theory, ethyl alcohol is formed by the reduction of acetaldehyde, the latter resulting from the decarboxylation of pyruvic acid. Although ethyl alcohol was not formed from pyruvic acid fermented by lactic acid bacteria, it is probable that conditions in the fermentation of glucose were such that they brought about the formation of ethyl alcohol from intermediary pyruvic acid.

The addition of certain hydrogen acceptors to fermentation of glucose may divert the normal course of dissimilation and thereby throw some light on the dissimilative mechanism. Acetaldehyde and acetylmethylcarbinol added to fermentations of glucose by heterofermentative bacteria resulted in a decrease in ethyl alcohol, lactic acid and glycerol and an increase in acetic acid and carbon dioxide. The added hydrogen acceptors were reduced, the acetaldehyde to ethyl alcohol and the acetylmethylcarbinol to 2,3-butyleneglycol. Added hydrogen acceptors may be expected to compete with hydrogen acceptors formed by the dissimilation of glucose. It appears that ethyl alcohol, lactic acid and glycerol are formed by the reduction of intermediary hydrogen acceptors.

Levulose plays two roles in fermentations by the heteroforms; (a) part is fermented to acetic acid, carbon dioxide, lactic acid and ethyl alcohol, and (b) part acts as a hydrogen acceptor and is reduced to mannitol. If the quantity of levulose changed to mannitol is subtracted from the total quantity of levulose fermented and the products calculated on the basis of the difference, the relationships are very similar to those obtained by fermentations of glucose to which hydrogen acceptors have been added.

The present work may be interpreted as supporting both the generally accepted scheme of dissimilation among bacteria involving methylglyoxal as a 3-carbon intermediary and an adaptation of the Embden-Meyerhof scheme of muscle glycolysis in which phosphoglyceric acid and pyruvic acid play an important part. The data obtained do not permit acceptance of one scheme to the exclusion of the other. In view of the many mechanisms that may bring about the end-products in the same quantitative relationships, and the isolation of intermediary compounds that do not satisfy a single scheme of dissimilation it is probable that no one of the present schemes governs fermentation under all conditions. Further study should conciliate the various theories of carbohydrate breakdown and clarify the roles played by intermediary compounds such as methylglyoxal and phosphoglyceric acid.

# THE EFFECT OF PHOSPHATE FERTILIZERS ON SOIL REACTION[1]

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Experiments were carried out to study the effect of different phosphate fertilizers on the reaction of Carrington loam, Carrington silt loam, Tama silt loam and Grundy silt loam. The soils were sieved, treated with different phosphate fertilizers, mixed well, potted and kept in the greenhouse under uniform conditions. Samples were taken periodically for laboratory analysis.

An analysis of variance of the data for 10 samples of the Carrington loam taken over a period of eight months showed that 120 pounds per acre of superphosphate decreased the pH to a highly significant extent. Two hundred forty pounds per acre caused a highly significant lowering of pH below that resulting from the 120 pound application. One thousand pounds per acre of rock phosphate increased the average pH above that of the untreated soil by an amount greater than the least mean difference which could be considered highly significant and 2,000 pounds per acre resulted in a further highly significant increase in pH. The exchangeable hydrogen was highly significantly correlated with pH, the correlation coefficient being —.9644. No significant difference was found in the base exchange capacity of the variously treated soils.

It is interesting to note that although the increase in mean pH from 5.79 to 5.85 as the result of applying 2,000 pounds of rock phosphate per acre was highly significant statistically, it was still very much below the pH value of 7.00 produced by 4,000 pounds of limestone, the amount needed to neutralize this soil according to the Truog test.

A second study was made of the effect of rock phosphate and sodium phosphate, each applied alone and in combination with lime, and of lime alone on the reaction of Grundy silt loam in the greenhouse. A statistical analysis of the data from 13 samplings of duplicate pots taken over a period of 20 months in which the soils were uniformly watered but not cropped showed that 500 pounds or more per acre of rock phosphate decreased the acidity by a highly significant quantity as measured by pH and by the Hardy and Lewis lime requirement method. Higher rates of rock phosphate seemed to be more effective in neutralizing acidity of the soil. However, rock phosphate did not produce a significant difference in the amount of exchangeable hydrogen in Grundy silt loam or in the base exchange capacity.

In order to get some idea of the effect on plant growth of the neutralizing value of rock phosphate a sweet clover crop was grown in a series of pots of Grundy silt loam treated the same as in the fallow experiment described above. Applications of 500 pounds per acre or more of rock phosphate resulted in highly significant increases in yields of sweet clover, the two highest rates of application, namely, 2500 and 3000 pounds per acre,

[1] Original thesis submitted June, 1936. Doctoral thesis number 365.

produced yields significantly greater than those obtained at lower rates of application.

An attempt was made to evaluate the variables causing these differences in yield following applications of rock phosphate. Besides the measurements of reaction, measurements were made of the amounts of exchangeable calcium, available phosphorus and nitrate nitrogen in the soil and of the percentage of calcium in the sweet clover plants.

High exchangeable calcium content of soil was associated with the high lime applications but no significant variability in exchangeable calcium within the lime series or within the unlimed series could be associated with differences in the amounts of phosphates used. This fact, together with the high response of sweet clover to sodium phosphate, which added no calcium to the soil, and the lack of significant differences in the calcium content of sweet clover grown on soils with the different amounts of rock phosphate added, showed that not enough exchangeable calcium was added by rock phosphate to be a factor in sweet clover culture.

Plotting available phosphorus against yields of sweet clover shows an association between available phosphorus content of the soil, yield of sweet clover and the amount of phosphorus applied wherever the same kind of phosphate was used and where the soils did not vary much in reaction. However, there was no significant correlation between yield and the amount of available phosphorus in the soil when the data for the whole experiment were considered.

That phosphorus alone was not the most important factor in yield of sweet clover is shown by the high yields obtained on soils of high pH but of very low phosphorus content and by the low yields of some soils with high available phosphorus content. On the other hand, pH seems to be very important in sweet clover culture. The highest yields were obtained on soils that had the highest pH.

A multiple correlation of yield of sweet clover with pH, exchangeable calcium, nitrate nitrogen and available phosphorus content of the soil and calcium content of the crop showed that pH was the only one of these variables which correlated significantly with yield. This fact, together with the general occurrence of higher yields where there were increases in pH values, indicates that the neutralizing effect of rock phosphate may be responsible in at least a small way for the increases in yield of sweet clover resulting from its use as a fertilizer.

The scope of the work was broadened in a third experiment to determine the effects of five different phosphate carriers on the reaction of three different acid soils as measured by pH. The soils used in this experiment were Grundy silt loam from southern Iowa, Tama silt loam from eastern Iowa and Carrington silt loam from northeastern Iowa. They were selected as being representative of the large areas of acid soils in those sections of the state. Because of the recent interest in the more concentrated phosphtaes, treble superphosphate, Ammo Phos "A" and ammoniated phosphate were used in comparison with rock phosphate and superphosphate. Three hundred pounds of superphosphate and equivalent amounts of ammoniated phosphate and of Ammo Phos "A" resulted in highly significant increases in the acidity of these three soils as measured by pH. Larger amounts of these materials caused further increases in acidity. Rock phosphate decreased the acidity of these soils to a highly

significant amount as also did treble superphosphate, although not to as great an extent as did the rock phosphate. Larger applications of these materials caused further decreases in acidity.

The effect of the different phosphate fertilizers on soil reaction was found to vary with soil type.

# THE DISSIMILATION OF CARBOHYDRATES BY THE COLON-AEROGENES BACTERIA[1]

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Although an extensive literature dealing with the dissimilation of carbohydrates by the colon-aerogenes group is already in existence, results as reported are frequently highly contradictory. The present investigation was undertaken with the object of gaining some further insight into the mechanism of carbohydrate dissimilation by that group of organisms.

In attempting to attain that objective, the following methods of attack were used:

1. The periodic analysis of fermentations with the determination of the relationships between substrate and products throughout the course of the fermentation.
2. The determination of the effect of varying conditions upon the ratios of fermentation products.
3. The comparison of the products formed from the dissimilation of different carbohydrates (glucose and xylose).
4. The action of the organisms upon compounds which they normally produce from glucose, both alone and in the presence of a fermentation of the sugar.

The experimental results show no significant differences between the mode of action of typical *Escherichia coli* and the M. R. positive, citrate positive, coli-aerogenes intermediates, upon glucose. Aeration of fermentations of glucose by *Esch. coli* results in the production of detectable quantities of acetylmethylcarbinol, and the accumulation of pyruvic acid as an end-product. Evidence indicates that the accumulated pyruvic acid does not result from the oxidation of lactic acid, but rather from the intervention of oxygen as a hydrogen acceptor preventing the normal reduction of intermediately formed pyruvic acid to lactic acid.

The periodic analysis of fermentations of glucose by *Esch. coli* and *Citrobacter freundii* demonstrates the tendency of those fermentations to change from a predominantly ethyl alcohol-acetic acid producing initial system to a system finally producing chiefly lactic acid. The same fact has been previously noted by Grey (1). The same method of attack shows that the dissimilation of glucose by *Esch. coli* is not a mechanism readily adaptable to explanation by a simple equation or series of equations. The fact that a constant ratio between substrate and products could not be obtained can only be explained as the result of variations in the rate of conversion of glucose to the given products, or to further conversions on these products, or to a combination of the two effects. The data obtained

[1] Original thesis submitted July, 1935. Doctoral thesis number 336.

by periodic analysis of fermentations and of fermentations of glucose in the presence of added organic acids, indicate that both acetic and succinic acids can undergo further conversions, probably through a system of reactions analogous to the Thunberg-Wieland series (2), and thus play the role of intermediary compounds. Suspensions of *C. freundii* produce pyruvic acid and carbon dioxide from succinic acid under conditions of aeration.

The fermentation of xylose by *Esch. coli* led to the production of lactic acid in approximately the molar equivalent of the xylose fermented. The data indicate that the preliminary attack on the pentose molecule consists of a cleavage into 3- and 2-carbon compounds, the three carbon fraction being subsequently converted to lactic acid. From xylose, much larger quantities of succinic acid were produced than from glucose under the same conditions. The above facts suggest that succinic acid owes its formation to the condensation of some 2-carbon intermediary rather than to the 2- and 4-carbon cleavage of hexose molecules as suggested by Virtanen (4) and by Scheffer (3).

*Esch. coli* reduces acetylmethylcarbinol to 2,3- butylene glycol in the presence of a fermentation of glucose. Evidence indicates that the reduction involves the same enzyme system and source of hydrogen as those causing the reduction of intermediately formed acetaldehyde to ethyl alcohol.

Although the results obtained under some conditions can be fitted to a reaction scheme such as that proposed by Scheffer (3), results under varied conditions and many data reported in the literature cannot be explained by such relatively simple mechanisms. Evidence available at present is not sufficiently conclusive to justify the acceptance of any one scheme of dissimilation in explanation of the mechanism of the conversion of glucose to the products of its fermentation by *Esch. coli.* Two or more alternatives with equally good experimental basis can be given in explanation of the formation of each end-product and probable intermediary compound. The fermentation appears to be a highly complicated system in which both the rates of formation of the various products from glucose and the rates of their further conversion, are continuously changing.

A periodic study of the fermentation of glucose by *Aerobacter indologenes* shows that acetic acid accumulated during the early phase subsequently undergoes a pronounced decrease, thus displaying the characteristics of an intermediary compound. The production of ethyl alcohol is linear with respect to the sugar fermented, as is also the sum of 1-carbon compounds, i. e., formic acid and caron dioxide, indicating that the decrease in acetic acid was not the result of its conversion to any of the latter compounds. The facts that 2,3-butylene glycol and acetic acid show a reciprocal relationship and that the sum of one-half the acetic acid with acetylmethylcarbinol and 2,3-butylene glycol gives a linear function, suggest that acetic acid is reduced and condensed to give 2,3-butylene glycol. Substantiation of the above conception is found in the demonstration that acetic acid added to a fermentation of glucose by *A. indologenes* is utilized and that its disappearance is accompanied by an increased yield of 2,3-butylene glycol and the absence of hydrogen in the evolved gas.

The increased yield of 2,3-butylene glycol in the presence of added acetate is approximately equivalent to one-half of the utilized acetic acid.

Acetate alone in a peptone medium was not reduced by *A. indologenes* in the presence of an excess of hydrogen. When an excess of hydrogen was supplied to a glucose-acetate fermentation, the quantity of acetic acid reduced corresponded with that reduced when extra hydrogen was not supplied. Under the conditions used, then, *A. aerogenes* does not activate molecular hydrogen for the reduction of acetic acid. Some more active form, possibly atomic hydrogen or an active hydrogen donator, must be necessary for that reduction.

The fermentation of xylose by *A. indologenes* leads to products comparable with those produced from glucose. From xylose, however, appreciable quantities of succinic acid were produced while that acid was not found among the products of the fermentation of glucose. Results suggest that succinic acid may play an intermediary role in the fermentation of xylose by *A. indologenes*.

Succinic acid is utilized by *A. indologenes* in the presence of glucose.

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# A METHOD OF QUANTITATIVE CHEMICAL ANALYSIS USING A PHOTON COUNTER[1]

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The successful application of spectrum analysis to quantitative determinations depends upon an accurate measurement of the intensity of the characteristic spectrum line or lines of the element considered. In the work on this problem a photon tube was used to measure the intensity of the 2347A beryllium line as different amounts of the latter element were introduced into an arc between two graphite electrodes. The light from this arc was passed through a Gaertner single prism, constant deviation type of quartz monochromator.

Several types of photon tubes were constructed similar to those described by Rajewsky (1) and Locher (2). The tube used in most of the work is similar to that of Locher. The Pyrex tube with a quartz window and a silver cathode is filled with 8 cm. of helium gas. A metal case surrounding the tube prevents stray light from entering.

The potential required for the field of the counter tube is furnished by a direct current power pack similar to that described by Schmitt (3). The power transformer has a maximum potential of 2,000 volts across the secondary. Half-wave rectification is obtained by an 866 mercury vapor rectifier. The filter system consists of two 300 henry, 15 ma. chokes and three 1 m.fd. condensers. All transformers and condensers are insulated for 2,000 volts. Voltage fluctuations are reduced by use of a '57 tube as a voltage regulator. Fluctuations in voltage are negligible even for line voltage variations of 10-15 volts. The output voltage can be varied from zero to nearly 2,000 volts.

The impulses from the photon tube are very small, making it necessary to have a sensitive amplifier to detect and record them. Several types of high gain amplifiers were tried. The one finally adopted is a modification of that used by Locher (4). The amplifier consists of a two-stage resistance-coupled circuit. A high gain 257 tube was used in the first stage and a 2A5 in the second stage. The latter was used to drive the speaker and the counting mechanism. The photon counter is connected to the amplifier by a 10$\mu\mu$.fd. condenser. A 300 megohm resistor is connected in series with the photon tube and the high voltage power pack. A filter system for the grid bias resistor is found necessary to reduce "feed back" into the 257 tube. The screen and plate circuits are also well filtered. The power supply for the amplifier, including transformers and filter system, is removed to a distance to prevent the amplifier from being affected by induced currents and mechanical vibrations. All leads from the high voltage source, photon tube and power pack are shielded and the shield grounded. By having the speaker connected in the plate circuit of the 2A5 the impulses passed on to the counting mechanism may be noted.

[1] Original thesis submitted June, 1936. Doctoral thesis number 374.

Since the impulses from the amplifier are at times more rapid than can be resolved by the mechanical recorder, it is necessary to have some means of reducing the number of counts per unit of time to such a value as can be recorded. The Wynne-Williams (5) "Scale of Two" thyratron counting circuit offers such a mechanism, since the impulses entering the counter are reduced by a "scale of two" for each pair of thyratrons employed. In this counting mechanism, a modification of the Wynne-Williams circuit is used. Four thyratrons are used, thus requiring four impulses from the amplifier to complete the cycle in the last pair of thyratrons. The mechanical recorder used is a modified form of that suggested by Van den Akker (6) for counting impulses from a Geiger-Müller tube. It consists of two permanent magnets between whose poles a small vibrator of transformer iron is placed. The vibrator is prevented from touching the poles by two small brass stops. Around the vibrator is placed a helix composed of two separate coils of wire. These coils are wound simultaneously and contain the same number of turns. Each of these coils is connected in the anode circuit of one of the last pair of thyratrons. As first one and then the other of the last pair of thyratrons is lighted the vibrator is driven from one pole to the other of the permanent magnet. The movements of the vibrator are recorded by a stop watch. The balance wheel of the watch is removed and the end of the vibrator connected to the escapement lever of the watch.

By means of a capillary pipette, a certain volume of a solution containing a known concentration of beryllium per cubic centimeter is introduced into a specially prepared cup in the lower graphite electrode. After evaporation, the electrode is placed in its holder and adjustd to a specified position by an optical focusing arrangment. Adjustment of the voltage across the photon tube to a standard value is accomplished by checking the counting rate produced by a standard radium sample. The arc is struck by using a graphite rod. Simultaneously the watch used for timing is started. The counting mechanism will start as soon as light strikes the photon tube. The period of excitation can be determined in two ways. One may either select a certain time, for example 2 minutes, for all excitations, or may continue until the entire electrode cup is burnt. With the type and size of electrodes used these two methods give quite similar results. The time required for complete burning is about two and one-half minutes. The usual procedure is to run several blank electrodes both before and after those containing the samples. The blank electrodes are prepared in exactly the same manner as those containing the beryllium samples. Thus one obtains a count from the blanks which is characteristic for all the electrodes of that type. The actual count registered by the counting mechanism is corrected by subtracting the count obtained for the blank electrodes. This corrected count is plotted against milligrams of beryllium present. The graphs obtained represent the variation of the number of counts with the number of milligrams of beryllium present upon the electrode.

Eight different test runs were made and the results plotted. The results of one of these runs is shown in the following table.

Run No. 3 — Electrode Type A
Time—2 Minutes — Treatment—Total Lacquer

| Milligrams Be | Counts | | Counts | | Counts | |
|---|---|---|---|---|---|---|
| | Actual | Correct | Actual | Correct | Actual | Correct |
| 0.10 | 225 | 118 | 223 | 116 | 231 | 124 |
| 0.05 | 206 | 99 | 209 | 102 | lost | lost |
| 0.03 | 178 | 71 | 190 | 83 | 190 | 83 |
| 0.01 | 146 | 39 | 141 | 34 | 142 | 35 |
| Blank | 107 | 107 | 107 | 107 | 107 | 107 |

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# THE CHEMICAL TRANSFORMATION OF ALIPHATIC ACIDS IN THE COURSE OF THE BUTYL-ACETONIC FERMENTATION[1]

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The chemism involved in the butyl-acetonic fermentation has been the subject of considerable research and speculation. The mechanism for the transformation of carbohydrates to the end products must necessarily be very flexible to account for the variation in ratios of the different products formed under varying conditions, the constancy of the same end products with a number of utilizable substrates, and the shifting nature of the fermentation in its different phases. There are several general methods for testing the probability of a reaction scheme in a fermentation process. In one procedure an isolation of the intermediates is attempted. However, the isolation of a given compound does not offer conclusive evidence that it is an intermediate unless it can be definitely established that the compound is a precursor of one or more of the end products. Another approach concerns the addition of non-proliferating cells or cell preparations to solutions of possible intermediates. A third procedure is the addition of the supposed intermediates to an active culture of the organism. The value of transfusing a postulated intermediate to an active fermentation as a means of gaining an insight into a fermentation mechanism is based upon the premise that the substance is fermentable and is subject to the same chain of reactions that occurs in the normal fermentation. It should also be kept in mind that the amount of a transfused chemical which the organism is able to utilize is an important consideration. An intermediate, when added to an active culture, should be converted quickly, completely, and in large amounts to its purported end products.

The purpose of the present study was to obtain further information concerning the chemical changes taking place in the butyl-acetonic fermentation. The line of attack pursued consisted in the addition of certain aliphatic acids to an actively growing culture of the butyl organism with subsequent analysis of the end products of the fermentation to determine the fermentability of the added acids and their course of chemical transformations.

As different cultural conditions bring about changes in the chemical processes involved, a first and most important caution was to guard against undue alterations from the normal course of fermentation. This led to the development of a procedure which permitted transfusion of various compounds to corn mash media with minimum deviations from controls. The organisms were grown in five per cent corn mash and in each experiment the initial inoculation was made from a stock spore culture kept on sea sand.

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From the experimental data it was found that *n*-butyric acid was transformed into *n*-butanol and to a somewhat lesser extent into acetone. The producion of carbon dioxide was increased and that of hydrogen decreased.

Acetic acid was converted almost entirely into acetone.

*n*-Butyric-acetic acid mixtures gave optimum yields of solvents when the ratio was 2:1. This mixture also resulted in yields of all solvents; the major transformation was to n-butanol with least conversion to ethanol.

Formic acid, even when added to an extent of less than four-tenths of a gram per liter of five per cent corn mash, proved toxic to the organism.

The transfusion of n-propionic acid resulted in the formation of a small amount of *n*-propanol together with acetone.

Evidence was obtained indicating that isobutyric acid was partially reduced to isobutyl alcohol.

The pH levels of a series of fermentations were varied from 3.8 to 5.3 by the addition of HCl or NaOH. Except for the fermentation carried out at a pH of 3.8, the solvent yields were fairly uniform although there was a slight tendency for increased acetone and *n*-butanol production where the pH was slightly more acid than that of the control.

The highest tolerance exhibited by the organism toward the transfused acids was for *n*-butyric acid, which could be added to an extent of three and one-half grams per liter of five per cent corn mash.

The results of this investigation tend to show that the butyl organism may produce varying quantities of one, two, or all three of the different solvents from the various transfused compounds. From this, we have the choice of several conclusions:

1. The transfused products may be direct intermediates in the fermentation, and are procursors to the given solvents as postulated in the different proposed mechanisms.

2. The transfused assumed intermediates may undergo the transformations assigned to them in the various mechanisms, but the course of the fermentation may be so altered that apparent conversion to other solvents occurs. This explanation would account for the apparent transformation of *n*-butyric acid into acetone.

3. The transfused substances may be regarded simply as fermentable substrates, and the solvents derived from a series of complex reactions involving the synthesis of *n*-butanol, acetone, and ethanol.

While these acids are fermentable, the limited tolerance displayed by the organism toward them does not seem to justify the conclusion that they are the sole intermediates to acetone and *n*-butanol.

# GASTRIC DIGESTION OF SOYBEAN FLOUR WHEN USED AS A SUBSTITUTE FOR COWS' MILK IN FEEDING DAIRY CALVES[1]

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One of the most serious problems with which the dairy farmer is concerned is the economical rearing of calves. A vast amount of work has been done to produce calf feeds which may displace milk in the calf ration, many stations having been outstanding in their interest in calf meals and gruels. The problem has usually been attacked either (1) by attempting the use of milk products such as dried and semi-dried skimmilk and buttermilk, or (2) by using cereals, packinghouse by-products, sugar, starch, etc., in the making of meals or gruels.

The use of soybean products in the rations of mature animals, and even for human consumption, has been quite extensive. Their use for human consumption among the poorer classes in the Orient is probably more common than the use of wheat. The Chinese have made rather extensive use of "milk" made from the soybeans, in human infant feeding. This suggested the use of soybean "milk" to replace cow's milk in feeding dairy calves. Accordingly a series of experiments was inaugurated to determine the rate at which soybean flour, fed as a gruel, passes from the abomasum of the calf. The secretion of gastric juice, which was used as a means of determining the rate of passage of the soybean flour from the stomach, was measured by (1) determining the volume of pure gastric juice secreted in Pavlov pouches, and (2) by determining the acidity of the stomach contents by use of rumen fistulae.

Three series of trials were conducted:

1. A series of twelve-hour trials using a test meal of one liter of soybean milk or whole cow's milk, fed after a fasting period of twenty-four hours.

2. A continuous trial of fourteen days, comparing soybean milk and whole cows' milk, with regular feedings of three to four pounds at eight-hour intervals, with seven days on soybean milk and seven days on cows' milk.

3. A series of sixteen-hour trials comparing test meals of one liter of skimmed cows' milk to one-half liter of "fortified soybean milk" (described below), fed after a fasting period of twelve hours, preceded by twelve hours on oatmeal gruel, in which (a) volume of gastric secretion was determined, and (b) free and total acidity of gastric contents were determined by titration, in calves with rumen fistulae.

The soybean milk used in series 1 and 2 was a mixture of 1 part soybean flour in 9 parts warm water. The "fortified soybean milk" was made by mixing fresh skimmilk with soybean flour so that one-third of the dry matter of the mixture came from skimmilk and two-thirds from soybean

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flour. This mixture contained 20 per cent dry matter. Eight cubic centimeters of a 40 per cent solution of calcium chloride per liter was also added to aid in coagulation. The whole milk used had a fat content ranging from 2.69 to 3 per cent and a curd tension of 80 to 95 grams. Skimmilk used as a check in the sixteen-hour trials, as well as a solvent for the soybean flour, came from the same cow.

The gastric acidity deetrminations were made by titration with N/100 NaOH, using Töpfer's reagent and phenolphthalein as indicators, for free and total acidity, respectively.

The results of the twelve-hour series in which four calves were used showed that, during the twelve-hour period, the volume of secretion on soybean milk was 11.87 per cent higher than that on whole cow's milk. During the first six hours of the twelve-hour period the secretion on soybean milk was 21.41 per cent higher than that on whole cow's milk, while for the second six hours the secretions caused by the two feeds were virtually equal. Moreover, the maximum secretion (largest volume of juice secreted in one half-hour period) was in every case larger when the soybean ration was fed, this increase averaging 39.35 per cent. These results indicate that the soybean diet functioned as a stronger secretagogue in the stomach, and that it left the stomach more rapidly than whole cows' milk.

In the continuous series the total volume of gastric secretion was slightly less when soybean gruel was fed than when whole milk was used. It is thought this reversal of results was due in part at least to the fact that in this series the calves were full-fed and that the volume of juice secreted per unit of protein material was less under these conditions. Moreover previous experiments show that much of the test meal spills into the rumen instead of all of it going directly into the abomasum. This may have affected the secretagogic capacity of the soybean material.

The results of the sixteen-hour trials show that the "fortified soybean milk" behaved somewhat like the skimmilk with which it was compared, for the volumes of secretion were near one another, although in all cases the secretion on soybean gruel was slightly larger. The volume of juice produced during the last half of the experimental period, when soybean milk was fed also increased 3.28 per cent over that when the skimmilk was fed, which indicated that the soybean material remained in the stomach for practically the same length of time as did the skimmilk. The secretion during the first half of the period was 7.71 per cent larger when soybean milk was fed than with skimmilk.

The results of determinations of acidity of the stomach contents when soybean milk and skimmilk were fed, showed that in all cases the soybean milk evoked on the average 12.58 per cent greater acidity in gastric contents than did skimmilk. On the other hand, the free acid was less by 19.2 per cent when the soybean milk was fed, than with skimmilk. Free acid appeared with the soybean diet four hours after feeding and with the skimmilk, two hours after feeding.

These results indicate that: Assuming that the volume of gastric secretion is in direct proportion with gastric digestion, then soybean flour, fed as in these trials, is digested in the calf's stomach at a slightly more rapid rate than either whole or skimmed cows' milk.

# STUDIES ON THE GROWTH AND REPRODUCTION IN THE RAT[1]

## (1) THE VALUE OF DIFFERENT COD LIVER OILS FOR REPRODUCTION

## (2) THE VALUE OF CERTAIN INDIVIDUAL FOODS AS SOURCES OF VITAMINS B AND G FOR GROWTH, REPRODUCTION AND LACTATION

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The vitamin E content of eight different brands of cod liver oil was investigated. The basis for comparison was the number of young born to females on diets in which these cod liver oils furnished vitamins A, D and E. No study was made concerning the possible oxidative destruction of the vitamins. One oil was shipped and stored in an iron container. This oil was the poorest from the standpoint of reproduction and its oxidative destruction was decidedly possible.

Diets containing cod liver oil No. I gave decidedly better reproduction than diets containing other brands of oil. No. II and No. III oils were of about equal value for reproduction, but were inferior to No. I. The No. IV brand was inferior to the three oils mentioned when comparisons were based on reproduction records. The oils known as No. V, No. VI and No. VII were of about equal value for reproduction, but were inferior to all other oils studied with the exception of No. VIII cod liver oil. The No. VIII oil permitted the least reproduction of any of the oils studied. It is significant that this oil was shipped in an iron container and that oxides of iron were present in the oil when this oil was withdrawn for use. The oxides of iron might have contributed to some oxidative destruction of the vitamins originally present in the oil.

Reproduction on diets containing five per cent of No. I cod liver oil as the sole source of vitamins A, D and E was compared with reproduction on diets containing five per cent of butterfat as the sole source of these vitamins. The diets containing the oil were uniformly better than those containing the butter, indicating a higher content of vitamin E in the oil.

Certain females on diets containing the different brands of cod liver oil were permitted to raise their young as a measure of their lactation ability. Diets containing No. I oil were the best for lactation. This superiortiy was shown by the lower mortality and the higher weaning weights of the young.

Diets containing No. I cod liver oil as the sole source of vitamins A, D and E were studied to determine whether yeast, wheat embryo or ether extracted wheat embryo was the best source of vitamins B and G for reproduction and lactation. Diets containing No. I oil and extracted wheat embryo were decidedly superior to diets containing either of the other sources of vitamins B and G. The yeast and unextracted wheat embryo were of about equal value for reproduction and lactation.

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Observed differences in reproduction were small throughout the study of the various cod liver oils. In every case the result on the experimental diets were compared with results using the diet employed for our stock colony. This was in the nature of a control over the experimental work. It soon became apparent that duplicate experiments run during different seasons of the same year or run during the same season of different years would not show identical results. This led to a study of reproduction and lactation in our stock colony. A study of these normal animals on a normal diet revealed the fact that variations in reproduction and lactation were larger than variations which had been considered significant in some previous experimental work. The study was continued for twenty-eight months and the number of young per female per month as well as the mortality of the young during each month showed the young per female in December, 1930, to be 0.22 while the young per female in June, 1930, appeared as 2.06. The mortality of the young in July of 1930 was 3.6 per cent while the mortality in December of 1930 was 64.3 per cent. These were the outside limits of the variations during the time of the investigation.

Purified diets, adequate in all respects except in vitamins B and G, were supplemented with various amounts of the diet used in our stock colony. Reproduction and lactation improved progressively with increases in the amount of the growing ration. This growing ration itself was improved by the addition of butterfat and was also improved by the addition of fish meal.

Reproduction and lactation were studied, using diets containing wheat as the sole source of vitamins B and G. These diets were further supplemented with various components of our growing ration. The components studied were tankage, buttermilk powder, linseed oilmeal, fish meal and alfalfa. The wheat diets were improved by the addition of every component with the exception of tankage. Buttermilk powder and fish meal were especially beneficial.

The vitamin B and G content of wheat, alfalfa, tankage, buttermilk powder, fish meal, linseed oilmeal and cottonseed meal was investigated in a series of lactation studies. Females from the stock colony were placed on the experimental diets at the time of parturition. These diets were adequate in all respects except in vitamins B and G and were supplemented with different percentages of each of the above components of our growing ration. The percentages used were ten, twenty-five, forty and sixty. Lactation was below normal on all of these diets studied. The twenty-five per cent level was selected for further study.

Females from the stock colony were placed on the diet which contained twenty-five per cent of the selected component as soon as the young were born. These females were each given certain vitamin supplements. The nature of these supplements and the amounts fed to each female daily are discussed below. The first supplement consisted of three-tenths of a gram of vitamin B adsorbate on fuller's earth. The second supplement consisted of one and one-half grams of vitamin G preparation from hog liver. The third supplement consisted of the vitamin B concentrate plus the vitamin G preparation from hog liver. The fourth supplement consisted of the vitamin B concentrate plus two and four-tenths grams of

autoclaved yeast. Success in lactation was judged on the basis of the mortality of the young and the weaning weights of the young.

These experiments indicated that the components deficient in vitamin B were tankage, buttermilk powder, linseed oilmeal, fish meal and possibly cottonseed meal. The components deficient in vitamin G were wheat, tankage, buttermilk powder, linseed oilmeal and cottonseed meal. Diets containing any one of these components, supplemented by vitamin B plus vitamin G, permitted lactation equivalent to the lactation on our growing ration. The vitamin G preparation from hog liver appeared to be better for lactation than autoclaved yeast.

# DISSIMILATION OF CARBYHYDRATES BY BACTERIA OF THE GENUS AEROBACILLUS[1]

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This investigation was undertaken with a two-fold objective: (1) to study the dissimilation of glucose and xylan and its derivative, xylose, by *Aerobacillus polymyxa* and *A. acetoethylicus;* (2) to investigate the formation and mutual relationships of acetylmethylcarbinol and 2,3-butylene glycol in fermentations.

Xylose is the hydrolytic product of xylan, which is an important component of such agricultural wastes as straws, oat hulls and cornstalks and cobs. A preliminary experiment showed that 43 to 63 per cent of xylan and 51 to 67 per cent of the pentosan content of corn cobs were fermented by *A. polymyxa*. Xylose in 1 per cent solutions was completely fermented. The acid hydrolysis of the xylan in agricultural wastes and the subsequent fermentation of the crude hydrolytic product may be of importance both to agriculture and to industry.

The medium used in these studies on the dissimilation of xylose and glucose consisted of 0.5 per cent $CaCO_3$, 0.2 per cent $K_2HPO_4$, 0.5 per cent peptone, 0.5 per cent yeast extract and 1 or 2 per cent carbohydrate. The addition of the yeast extract markedly increased the rate of fermentation, reducing the time for completion from 6 or 7 days to as low as 48 hours.

The products of the fermentation of xylose were found to be the same as those from glucose and in the same proportions. The fermentation of these sugars by *A. polymyxa* results in carbon dioxide, hydrogen, ethyl alcohol, acetylmethylcarbinol, 2,3-butylene glycol, and acetic, formic, lactic and succinic acids. *A. acetoethylicus* forms acetone and all of the other products mentioned except acetylmethylcarbinol (sometimes in traces) and 2,3-butylene glycol.

The fermentation of xylose by *A. acetoethylicus* in a medium containing $NaHCO_3$ results in much more formic and acetic acids and less acetone than in a similar medium containing $CaCO_3$. In one experiment with $NaHCO_3$ 26.7 per cent of the fermented xylose was represented by formic acid, 30.6 per cent by acetic acid and 0.1 per cent by acetone, while a similar experiment with $CaCO_3$ resulted in 4.6 per cent of xylose as formic acid, no acetic acid and 11.1 per cent acetone. *A. polymyxa* forms more formic and acetic acids and less 2,3 butylene glycol from xylose with $NaHCO_3$ than when $CaCO_3$ is used. The difference in yields is probably the result of the differences in the pH of the media. $CaCO_3$ buffers the medium at a pH of 5.8 to 5.9 and $NaHCO_3$ at approximately 6.5 to 6.6. Osburn (1934) found that free acetic acid exists only below pH 6.3 in solutions buffered with $K_2HPO_4$-$KH_2PO_4$ mixtures. Above pH 6.3, the

[1] Original thesis submitted August, 1935. Doctoral thesis number 346.

salt of the acid is present in the medium and it fails to undergo further reaction. The same is likely true for formic acid.

The addition of acetic acid to a $CaCO_3$ buffered glucose containing medium which is fermented by *A. Polymyxa* results in an increased yield of 2,3-butylene glycol plus acetylmethylcarbinol and a decreased yield of hydrogen. Since less acetic acid is present in the fermented liquor than what was added, some was converted to acetylmethylcarbinol and 2,3-butylene glycol. Probably the acetic acid was reduced to acetaldehyde which was then condensed to acetylmethylcarbinol and the latter reduced to the glycol. The addition of acetic acid to a fermentation of glucose by *A. acetoethylicus* increases the acetone and ethyl alcohol and decreases the hydrogen produced. Thus it appears that acetic acid serves both as an intermediary and as an end product in fermentations by *A. polymyxa* and *A. acetoethylicus.*

The evidence that acetaldehyde is an intermediary in the fermentations of glucose by *A. polymyxa* is two-fold: (1) Added acetaldehyde increases the yield of ethyl alcohol, acetylmethylcarbinol, and 2,3-butylene glycol, and (2) the aldehyde was fixed in normal fermentations of glucose by the use of both $CaSO_3$ and $NaHSO_3$. The melting point of the dimedon derivative was 138° C. The acetaldehyde dimedon derivative melted at 139° C. and the mixed melting point was 138° C.

Acetylmethylcarbinol ($CH_3 . CO . CHOH . CH_3$) and 2,3-butylene glycol ($CH_3 . CHOH . CHOH . CH_3$) are products of the fermentation of glucose and xylose by *A. polymyxa.* An investigation of the production and mutual relationships of these two compounds was extended from *A. polymyxa* to citric acid fermenting streptococci and the butyl-acetone group of microörganisms.

Little acetylmethylcarbinol accumulates during the fermentation of glucose by *A. polymyxa* even when oxygen is bubbled through the culture. 2, 3-Butylene glycol is produced rapidly and added acetylmethylcarbinol is reduced to the glycol when considerable sugar is present and the oxidation-reduction potential is low. When all the sugar has been fermented and the redox potential has risen considerably, 2, 3-butylene glycol will donate hydrogen to oxygen with the formation of acetylmethylcarbinol. Thus, either compound may serve as the precursor of the other in fermentations.

After a maximum yield of acetylmethylcarbinol plus diacetyl ($CH_3.CO.CO.CH_3$) has been obtained in butter cultures, continued holding of the cultures frequently results in a decrease of these substances. The citric acid fermenting streptococci in the cultures apparently are responsible for the decrease. Studies were made to determine the fate of the carbinol and diacetyl in various cultures of these organisms. When added to a tomato bouillon or milk culture of one of the citric acid fermenting streptococci, acetylmethylcarbinol is partially reduced to the corresponding glycol. The method employed was to inoculate the medium and then incubate for 24 or 48 hours to obtain good growth. Acetylmethylcarbinol, or diacetyl, was then added and determinations for the carbinol or diacetyl and 2, 3-butylene glycol were made at once and after various periods of holding. Typical results are given. The molarity of acetylmethylcarbinol in a tomato bouillon culture of organism 29 was reduced from a molarity of 0.0052 to 0.0003 in 72 hours. During this time

the molarity of 2, 3-butylene glycol increased from 0.0006 to 0.0058. In a tomato bouillon culture of organism 49, added diacetyl was reduced from a molarity of 0.0025 to 0.0001 in 24 hours, while the molarity of 2, 3-butylene glycol increased from 0.0010 to 0.0031. The reduction of the carbinol in a tomato bouillon culture of one of the citric acid fermenting streptococci is hindered by reducing the pH to 3.8 by the addition of sulphuric acid. The addition of 0.5 ml. of 30 per cent $H_2O_2$ per liter of skim milk culture or 1.5 ml. of either acetaldehyde or propionaldehyde per 1200 ml. of culture markedly inhibited the reduction of the carbinol.

*Clostridium acetobutylicum* normally produces small quantities of acetylmethylcarbinol but is unable to reduce added carbinol to 2,3-butylicum glycol during a fermentation of glucose. *Cl. pectinovorum, Cl. butylicum* and *Cl. pasteurianum* do not form the carbinol in normal fermentations but are able to completely reduce added carbinol to the glycol. *A. polymyxa* and the citric acid fermenting streptococci are able both to form the carbinol and to reduce it to 2,3-butylene glycol. Since *Cl. acetobutylicum* forms butyl and ethyl alcohols by the reduction of the corresponding acids, the failure to reduce acetylmethylcarbinol suggests that a different dehydrogenase is required for the carbinol-glycol change than for the reduction of the acids.

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# BACTERIOLOGICAL STUDIES ON SOME DEFECTS OF CREAM CHEESE SPREADS[1]

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Within recent years the manufacture of cream cheese spreads has developed into an important branch of the dairy manufacturing industry. In general, cream cheese spreads consist of cream cheese to which various products are added to secure a variety of flavors; the spreads are heated to a relatively high temperature and placed, while hot, in the final container, which is often a vacuum sealed glass. In cream cheese spreads, like most other dairy products, certain defects have appeared which require bacteriological study in order to prevent recurrence and resulting financial loss to the manufacturer.

No work has been reported on the bacteriology of cream cheese spreads. A number of investigators have found anaerobic organisms present in various dairy products. Csiszar, in studying the bacteriological defects of process cheese, found that the organisms responsible for spoilage were *Bacillus sporogenes, Bacillus putrificus* and *Bacillus saccharobutyricus*. He found that these organisms could not be killed in process cheese by lowering the pH or adding salt or any other preservatives which he studied, without lowering the quality of the cheese.

## STUDIES ON GAS PRODUCTION IN CREAM CHEESE SPREADS

The outbreak of gas formation in cream cheese spreads which was responsible for this study occurred in the spring and summer of 1934. Gassiness was observed to a limited extent in several varieties of spreads but the defect was particularly noticeable in Roquefort type spread.

Only a small percentage of the jars in a batch developed gas. Under ordinary marketing conditions the cheese spreads were held at room temperature and at times remained normal for 2 or 3 months and then suddenly developed gas. When the spreads were held at 37° C. gassiness usually developed in 5 to 10 days if it developed at all. The gassy spreads had no off flavor or odor and appeared normal in every way except that gas was produced in the cheese. No typical gas holes were apparent, but the spread, inside the glass jar, would break and the upper portion would be forced from the lower portion with a clean break. The defect varied from only a few breaks in the cheese to the condition where approximately half of the spread was pushed out of the jar.

The organism responsible for the outbreak of gas formation was found to be a heat resistant anaerobic organism. The organism was very unusual in its growth requirements, since it could be grown only in peptone-litmus milk, in litmus milk to which a small amount of Roquefort type cheese had been added and in Roquefort type cheese emulsion.

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[1] Original thesis submitted June, 1936. Doctoral thesis number 389.

Nineteen cultures of the gas producing organism were isolated; 12 came from 12 different lots of defective Roquefort type cream cheese spread, 5 from domestic blue cheese and 1 each from Danish bleu and French bleu.

The gas producing organism was found to be very heat resistant in peptone-litmus milk at high pH values, but as the pH was lowered the heat resistance also was lowered. The addition of salt in concentrations over 2 per cent decreased the heat resistance of the organism at a given pH. Since Roquefort type cheese contains 4 to 5 per cent salt, the addition of enough acid to lower the pH to about 5.40, together with holding the cheese at 85° C. for 20 minutes, should enable the manufacturers to make Roquefort type cheese spread in which gas production does not occur, even though the organism is known to be present.

Since the organism was so heat resistant, it was placed in the genus Clostridium, although spores were never observed. The name *Clostridium peptophilum* is proposed. A complete description of the organism could not be prepared since it grew in only a few media.

## STUDIES ON LIQUEFACTION IN CREAM CHEESE SPREADS

About the time the outbreak of gassiness occurred, the same plant began having trouble with liquefaction in a few varieties of cream cheese spreads, especially the Roquefort and pineapple spreads. This defect developed in about 3 to 4 weeks at room temperature and in about 5 to 10 days at 37° C. Only a small percentage of the jars in each batch showed liquefaction. The defect occurred in all degrees, ranging from only a small amount of liquid on the surface to the condition where approximately half of the spread in the jar was liquefied. When liquefaction was extensive the liquid collected around the outside of the cheese spread and was translucent. In a few cases enough gas was produced to release the vacuum seal. When the lid was removed a very pronounced putrid odor was noted.

Liquefaction in cream cheese spreads was found to be due to the action of a heat resistant spore bearing organism that was identified as *Clostridium sporogenes.*

*Clostridium sporogenes* was not killed by heating at 95° C. for 20 minutes in litmus milk with a pH of 5.21 and a salt concentration of 10 per cent. It was possible to inhibit the digestion of the milk by combinations of high salt and low pH values, but in all cases the salt concentrations and pH values which inhibited digestion could not be used commercially without ruining the quality of the spread.

# I. THE RELATIVE AROMATICITY OF FURAN

# II. HEAVY HYDROGEN IN SOME NATURALLY OCCURRING ORGANIC COMPOUNDS AND MIXTURES

## I. THE RELATIVE AROMATICITY OF FURAN

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Furan has previously been shown to have pronounced aromatic properties by Gilman, *et al.* These investigators used the acid cleavage of furyl-aryl-lead compounds (1a), nitration of furyl aryl ketones (1b), Friedel-Crafts reactions on furan compounds (1c), and the metalation of furan compounds (1d).

In the present work competitive substitutions have been applied to compounds containing aryl and furyl nuclei.

The nitration of phenyl 2-furoate to yield phenyl 5-nitro-2-furoate (m. 121-1.5°) was perhaps the most significant evidence found for the pronounced aromaticity of furan. This fact will be more readily grasped when one considers that nitration of phenyl benzoate results in 4-nitrophenyl benzoate (2). Nitration of 3-methoxyphenyl 2-furoate (b. 179°/10 mm.) resulted in a mixture of 3-methoxy-6-nitrophenol and its furoic ester m. 106-6.5°. Nitration of diphenyl dehydromucate (m. 138.5°) gave picric acid only, while nitration of 2-naphthyl 2-furoate (m. 121-2°) gave a mixture which upon oxidation yielded 2-furoic acid. Acetylation of phenyl 2-furoate by means of stannic chloride and acetic anhydride in benzene solution was unsuccessful.

Bromination, nitration and mercuration of 2-furylphenylmethane and 1-(2)furyl-2-phenylethylene (m. 53-4°), prepared by decarboxylation of $\beta$-(2)furyl-$\alpha$-phenylacrylic acid, gave negative results, only tars and decomposition products being isolated. An excellent derivative of 2-furylphenylmethane was found in $\Delta^4$-3-benzyl-3,6-endoxytetrahydrophthalic anhydride (m. 102-2.5°), prepared by the action of maleic anhydride on the furylphenylmethane.

The sulfonation of 2-benzoylfuran gave 5-benzoylfuran 2-sulfonic acid (isolated as the barium salt). Bromination of 2-benzoylfuran gave 5-bromo-2-benzoylfuran (m. 37-7.5°; oxime, m. 139-40°), while bromination of 2-(*p*)anisoylfuran gave an addition product (m. 131-2°) which lost hydrogen bromide to yield 5-bromo-2-(*p*)anisoylfuran (m. 73-4°). Mercuration of 2-benzoylfuran resulted in highly-mercurated products in which the amount of mercury corresponded to no calculated values. Nitration of 2-(*p*)toluylfuran (b. 185.7°/18 mm.), prepared from toluene, 2-furoyl chloride and aluminum chloride, resulted in 5-nitro-2-(*p*))toluylfuran (m. 122-3°), and nitration of 2-(*p*)-anisoylfuran gave 5-nitro-2-(4)methoxy(3)nitrobenzoylfuran (m. 123-4°) even when reasonable pre-

[1] Original thesis submitted June, 1936. Doctoral thesis number 375.

cautions to effect mononitration were taken. 5-Nitro-2-(*p*)anisoylfuran (m. 126-7°) was prepared by replacing the bromine in 5-bromo-2-(*p*)anisoylfuran by means of nitrogen trioxide. Nitration of this compound gave the former dinitro ketone.

Attempts to prepare 2-(2)furoylpyridine by the action of 2-furylmagnesium iodide on 2-cyanopyridine were failures.

Nitration of 5-nitro-2-benzoylfuran was unsuccessful, and the nitration of 2,5-dibenzoylfuran gave the two compounds reported by Phelps and Hale (3). The compound melting at 193° was most likely 2,5-di(*m*)-nitrobenzoyl-3-nitrofuran, but the other compound (m. 129-30°) may have been a ring scission product. Analyses of the latter product were correct for a mononitrated compound, but oxidation gave only an acid (m. 132-3°) whose identity is as yet obscure. Mercuration of 2,5--dibenzoylfuran was unsuccessful.

Furonitrile apparently possesses some unorthodox properties since nitration to yield 5-nitro-2-furonitrile (m. 67.8°) had to be forced, while benzonitrile nitrates readily under mild conditions (4) and other negatively substituted furans nitrate (5) much easier than do their benzene analogs.

Several Friedel-Crafts reactions were performed using stannic chloride, acetic anhydride and the furan compound in toluene solution. 2,5-Dimethylfuran and ethyl 2-furoate acetylated readily with formation of only traces of *p*-methylacetophenone. Methyl 5-bromo-2-furoate gave *p*-methylacetophenone and recovered ester as the only products, while in benzene solution no actophenone was formed.

## SUMMARY

Nitration, bromination and sulfonation have been successfully applied to the relative aromaticity of furan. Furan is more aromatic than benzene, toluene and anisole and would probably lie close to anisole in a series of aromaticity on the basis of nuclear substitution.

Some indirect evidence concerning the furan *beta*-carbon has been presented.

## II. HEAVY HYDROGEN IN SOME NATURALLY OCCURRING ORGANIC COMPOUNDS AND MIXTURES

The natural abundance of deuterium has received a good deal of attention in the past few years. Unfortunately, several objections have arisen (6) which render worthless most, if not all, of the accumulated data. These criticisms revolve about the fact that previous investigators have neglected the difference between the atomic weights of commercial oxygen and that of normal oxygen, and the difference between the atomic weights of oxygen in the air and oxygen in water which amounts to six parts per million difference in the densities of water prepared from these two oxygens, the air-oxygen water being the heavier (6).

The combustions of fungi yeast, etc., offer some difficulty and this thesis presents a simple method of effecting adequate combustion of these substances.

Previously unpublished results of the determination of heavy water in beef tissues were also reported in this thesis. The particular tissues in-

vestigated were the hide, kidney, prostate, spinal cord, pancreas, thyroid, muscle, ovary, thymus, heart, aorta, testis, marrow, spleen, lung, brain, liver, liver water, blood (defibrinated), blood water, blood fibrin, haemoglobin, blood serum and tallow of adult cattle. This work was performed under the direction of Dr. Henry Gilman in collaboration with Dr. H. L. Keil, Dr. A. W. Ralston, Mr. V. Conquest, Dr. W. H. Jennings, Dr. W. E. Catlin and Mr. M. T. Kelley.

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# AN INVESTIGATION OF TYPES OR STRAINS OF THE MOSAIC VIRUS OF SUGARCANE IN LOUISIANA[1]

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The virus which causes the mosaic disease of sugarcane and related grasses has been divided into four strains based upon the symptoms produced on two different host varieties, C. P. 28/60 and Louisiana Purple. Strain 1 produces a pattern characterized by very mild chlorosis and no necrosis or stunting on C. P. 28/60; strain 2 produces severe and general chlorosis, varying amounts of necrosis, and pronounced stunting on the same variety; while strains 3 and 4 produce identical symptoms of severe chlorosis in the form of streaks and a necrotic condition that may or may not severely blight and sometimes even kill the growing point. Strains 1, 2 and 4 produce only ordinary, rather mild, symptoms of Louisiana Purple and several other commercial varieties, while strain 3 has approximately the same effect on all varieties as described above for C. P. 28/60.

Strains 2 and 4 have been obtained from almost all sections of Louisiana, although there seems to be a tendency for one or the other to predominate in certain areas, while strain 1 has been identified chiefly from Canal Point seedlings at the United States Sugar Plant Field Station at Houma and in one isolated area about 200 miles away. The occurrence of strain 3 has been limited to Rosewood Plantation, where it was found originally, with the exception of a very few isolated, single stools.

Evidence of the probable existence of additional strains of the sugarcane mosaic virus in Louisiana is rather plentiful. One virus source, that had been identified as strain 2, was observed to produce a new type of necrosis on the older leaves of infected plants of the variety Co. 281. Another source, that had been called strain 4, was also used to inoculate Co. 281. Cuttings from this material exhibited about 40 per cent "germination recovery" which, previously, had been practically non-occurring in this variety. These two instances indicate definite differences between each of these virus sources and the four described above and given numerical designations. Still a third possibility of a different strain is the sudden appearance, in a single cane field, of quite an appreciable mosaic infection on C. P. 807, a variety long considered immune to the disease. Identification of this source of the virus is, at present, held up by failure to obtain infection when the usual technique is employed. Similar difficulties have been encountered with juice from other resistant varieties and the discovery of a procedure for securing juice, comparable in infectiousness with that from susceptible varieties, from such varieties would be of great value.

Careful observations of symptom patterns on many varieties, in the field as well as in greenhouse inoculations, are being made with the pur-

[1] Original thesis submitted August, 1935. Doctoral thesis number 339.

pose in mind of discovering new differential host varieties that may aid in further resolving the present strains or, more likely, in differentiating other virus sources that are already suspected or known to be different from the present strains. The thousands of seedlings now available probably offer the greatest promise as new differential hosts because of the extreme heterozygosity represented in their respective genetic make-ups. Fortunately, since sugarcane is propagated only by cuttings, each clone can be preserved and any peculiar utility it might offer as a differential host, due to its heterozygous conditions, can be made permanently available.

The three P. O. J. varieties, 36-M, 213 and 234, were 100 per cent mosaic in most Louisiana cane fields in 1925. By 1930, the disease had almost entirely disappeared from all plantings of P. O. J. 213, and was materially lessened in the other two, particularly 36-M, by what may be called the "recovery process," which consists of a combination of both "foliage" and "germination recovery." The latter is probably of much greater importance although the former has been definitely shown to be operative at times. A wave of secondary spread, beginning in 1930 and gaining in intensity during the succeeding years, has again brought infection to approximately 100 per cent in these varieties. During the early part of this period both types of recovery were common in P. O. J. 36-M, the average germination recovery with pedigreed-mosaic cuttings was over 50 per cent, and occurred to lesser extents in P. O. J. 234. Certain lines of P. O. J. 36-M showed significantly more recovery than other pedigreed-mosaic lines. No recovery of either type, however, occurred with the newly-infected material of P. O. J. 213.

During this period before the demonstration of strains of the virus it seemed that the differential rates of recovery observed in P. O. J. 36-M could best be explained by the assumption of a qualitative attenuation of the virus and that P. O. J. 213 had been infected with a virulent source directly from wild grasses, to which had been ascribed the power to "step up" the virulence of the disease. Attenuation could not, however, be experimentally demonstrated and so the discovery of "strains" of the virus offered a new and apparently more tenable theory to explain this phenomenon. In other words, a variety infected with one strain may be able to throw it off and recover but if infected with a different strain would be able to produce only diseased offspring. The virus that infected P. O. J. 213, for instance, prior to 1925 was a different and, for this variety at least, a less tenacious strain than the one that infected this variety in 1930 and subsequently. Experiments are planned that will give definite information on this phase of the problem.

Sugarcane mosaic was probably introduced in the United States prior to 1913, but, until 1932, there had never been observed on any variety any greater variation in mosaic symptoms than could be ascribed to normal variation. There had, of course, been wide differences in symptom patterns on different varieties. Prior to 1913, when quarantine laws went into effect, promiscuous shipments of sugarcane cuttings had been received from all over the world and the same was apparently true of other sugarcane-growing countries. It seems unlikely, however, that the strains here treated were brought in from different countries and have maintained their identity ever since without detection. This is particu-

larly true of strain 3, which causes such severe symptoms on all varieties that it certainly could not have been present for so long without attracting some attention. It would also have been more widely distributed than it seems to be at the present time. A systematic survey and strain determination of the sugarcane mosaic virus in other countries that produce sugarcane, such as is now being conducted in Louisiana, would be invaluable in determining the possible source of individual strains as well as their world distribution. In view of the evidence as it appears now, it would seem that strains of the virus are arising locally, possibly within the cane plant or by passage through other gramineous hosts. These less-favored hosts, or the insect vector (*Aphis maidis* Fitch) or even some new vector, may be fractionating a compound virus that has hitherto existed as a mixture. This would not be without precedent among virus diseases of other plants. Mutations of a single original virus, assuming it to be a living entity, offers a further possible explanation.

# STUDIES ON INSECT HEMOLYMPH WITH SPECIAL REFERENCE TO SOME FACTORS INFLUENCING MITOTICALLY DIVIDING CELLS[1]

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Many conditions and substances have been reported to influence, favorably or unfavorably, multiplication of protozoa, of bacteria, of yeasts, of vertebrate and invertebrate embryonic cells, of normal and pathological cells in plants and animals, and of isolated tissue culture cells; but only four references to stimulation of mitosis of insect hemolymph cells have been found. In 1924 Paillot[3] injected *Euproctis chrysorrhoea* larvae with emulsions of *Bacillus melolanthae non liquefaciens* and found that dividing amebocytes rose from three or four per thousand cells to thirty or forty. In 1925 and 1927 Iwasaki[4, 5] used *Galleria mellonella* larvae in a study of the effects of temperature changes and injections of bacteria, peptone, albumin, and various vaseline emulsions on the mitotic counts. From a normal of one or two karyokinetic cells per thousand, counts as high as 136 per thousand were obtained with bacteria injections. He found also that cells other than hemolymph cells were not involved in the mitotic reaction. In 1933 Paillot[6] analyzed his data from many insects and discovered that cells which he called macronucleocytes were the type which responded to stimuli. He believes the "caryocinétose" reaction is connected in some way with the secretion of antagonistic immunity principles by the phagocytic macronucleocytes.

In the research referred to below, the roach, *Blatta orientalis*, was used as an experimental insect in a study of its hemolymph cell response to various conditions, to injections of a number of substances (enumerated later), and to inoculations with several different bacteria. Large numphs or adults, male or female, were selected randomly from stock colonies, kept under close observation for ten to fourteen days, and then used either as control or experimental animals.

Injections were made with a small syringe whose hollow needle was inserted through the coxa-femur conjunctival fold of a metathoracic leg. About one-twentieth cc. (approximately 10 per cent of the average body weight) was injected. Bacteria were introduced by inserting a dissecting needle, wet with the culture, into the body cavity through the conjunctival folds between the tergal plates of the abdomen, about midway between the mid-dorsal line and the side of the animal.

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[1] Original thesis submitted December, 1935. Doctoral thesis number 349.

[2] Aided by grants from the Rockefeller Fluid Research Fund, administered through Iowa State College.

[3] Paillot, Bull. Hist. Appl. à Physiol. et à Path., 1:216-223 (1924).

[4] Iwasaki, Ann. d. Physiol. et d. Physicochimie Biol., 1:580-621 (1925).

[5] Iwasaki, Arch. Anat. Micr., 23:319-346 (1927).

[6] Paillot, L'infection chez les insectes. 535 pp. Patissier, Paris (1933).

Hemolymph cell counts were made by the method described by Yeager and Tauber[7], except that 2,000 cells were counted for each determination.

## RESULTS

Approximately 250 insects were used. A total of 2,595 mitotically dividing cell (M.D.C.) counts, each of 2,000 cells, was made; consequently, summarizing percentages were based on a total of 5,190,000 counted cells. Counts of 2,000 cells were found by experiment to be the minimum number that could be used to give satisfactory and consistent results. Duplicate counts of 2,000 cells each on the same sample, or from different samples from the same animal, give data that agree within reasonable limits.

TABLE 1. *Summary of results from control hemolymph cell counts*

| Description of animals | No. of insects | No. counts made | Range Pctg. M.D.C. | Average Pctg. M.D.C. |
|---|---|---|---|---|
| ♂ Nymph control | 4 | 96 | 0.00-0.45 | 0.203 |
| ♂ Adult control | 3 | 67 | 0.05-0.40 | 0.186 |
| ♀ Nymph control | 4 | 145 | 0.00-0.50 | 0.194 |
| ♀ Adult control | 3 | 68 | 0.05-0.40 | 0.126 |
| Other controls, sex and age not checked | 16 | 192 | 0.00-0.40 | 0.172 |
| All controls | 30 | 568 | 0.00-0.50 | 0.186 |
| Initial count of all experimental animals | 124 | 124 | 0.00-0.45 | 0.196 |

Ranges of percentages of dividing cells for some other groups follow (obtained at room temperature unless otherwise noted): Ovipositing females, 0.05-0.40; molting animals, 0.00-1.00; at 5° C., 0.00-0.20; at 37° C., 0.20-1.30; injected with *Bacillus subtilis* suspensions, 0.00-0.40; injected with *Serratia marcescens* suspension, 0.00-0.35; inoculated with *Staphylococcus aureus*, 0.00-2.80; fed *Staph. aureus*, 0.05-1.50; natural coccus infection, 0.20-2.35; natural rod infection, 0.15-3.10; paralyzed animals, 0.20-1.85; animals with abnormally vacuolated cells, 0.35-2.05. Ranges of percentages following the injection of additional materials were: Standard bacteriological nutrient broth, 0.10-1.95; 5 per cent beef extract, 0.00-2.10; 10 per cent peptone, 0.00-1.60; chick embryo extract, 0.00-2.70; defibrinated rat blood, 0.00-1.20; 10 per cent hemoglobin (horse), 0.05-3.80; 10 per cent egg albumin, 0.35-0.70; 5 per cent aspartic or glutamic acids, glycocoll, alanine, or tryptophane, 0.00-0.55; 5 per cent cysteine, 0.00-2.50; 10 per cent glutathione, 0.00-1.10; 40U insulin, 0.30-5.30; 10 per cent dinitrophenol, 0.05-1.50; thyroxin (1 grain in 10 cc.), 0.05-1.90; 10 per cent glucose, 0.05-0.40; 1 per cent $PbCl_2$, 0.05-0.40; 1 per cent rotenone, 0.00-0.55.

Based on 5,190,000 cells, percentages of dividing cells in terms of mitotic phases are: prophase, 0.130; metaphase, 0.026; anaphase, 0.089;

[7] Yeager and Tauber, Proc. Soc. Expt. Biol. and Med., 30:861-863 (1933).

and telophase, 0.154. Percentage of amitotic cells was 0.0066; multinucleate (two to six nuclear fragments) cells, 0.026.

## DISCUSSION AND CONCLUSIONS

Considering the large amount of data collected from normal control animals and from preliminary control periods for experimentals, it seems evident that a normal specimen of *Blatta orientalis*, be it male or female, adult or large nymph, has a M.D.C. count within the limits of 0.00-0.50 per cent. Counts of 0.00 per cent (less than one in 2,000 cells) were listed 43 times in the 604 control determinations made; 0.50 per cent only three times. Controls kept over 132 days maintained counts within the above range. The slight hemorrhages connected with the sampling of the hemolymph evidently did not influence the mitotic count. The range of average values (from 0.116 per cent to 0.250 per cent) might lead one to suspect some factor or factors of influencing various specimens. However, high and low averages were found among males, females, adults, and large nymphs, without relationship to the sex and age factors mentioned, or to some other factors which were controlled.

Although no connection between daily fluctuations in counts and daily changes in room temperature was found, a low temperature of 5° C. did decrease the mitotic evaluation, and subjection to a continuous temperature of 37° C. resulted in a decided increase in count values.

Since Tauber and Yeager[8] had pointed out that high total hemolymph cell counts seemed associated with egg formation in crickets and other insects, a check was made to see if high M.D.C. counts were also an accompaniment of the condition. No deviation from the normal value was found, however. On the other hand, ecdysis, another physiological activity, is found to be associated with changes in dividing cell counts. Ecdysis is preceded by and accompanied by a distinct decrease in the percentage of mitotic cells. After molting the count tends to remain low for about a day, then increases, and usually reaches an optimum (as high as 1.0 per cent) on the third day. On the fourth day the count decreases and on the fifth or following days comes within the normal range. Reiche[9] has shown that tissue autolysates favor mitosis; in ecdysis the cytolytic products from tissue breakdown may contain some stimulating substance.

With the exception of *Staphylococcus aureus* this species of roach seemed quite resistant to the bacteria injected or inoculated into its hemocoele. Susceptibility to *Staph. aureus* was very pronounced, and multiplication of the invading organism was accompanied by a rise in the M.D.C. values. The infection was also associated with definite external and internal symptoms, often terminated by death. The body became convexly arched and hemolymph cells became "ragged" and highly vacuolated. In connection with this phase of the study, an interesting observation was made. After death due to causes other than bacterial infection, hemolymph of control or experimental animals would, in many cases, become filled with bacteria within a short time, though none had been seen before the termination of activities. Apparently a latent or controlled infection

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[8] Tauber and Yeager, Iowa State College Jour. Sci., 9:13-24 (1934).

[9] Reiche, Ztschr. f. Bot., 16:241-250 (1924).

had been present all the time and broke out as soon as the checking mechanism ceased to function.

Clues as to how natural bacterial infections may be spread were uncovered. Normal, uninfected animals given access to food soaked in cultures of *Staphylococcus aureus* often became diseased. Presumably the bacteria entered the hemolymph either through the gut wall, or through breaks in the exoskeleton. When one remembers the roach's cannibalistic habits, it is clear how diseases may pass from one animal to another.

Symptoms in those animals suffering with either of the two naturally occurring bacteria are much the same as indicated for *Staphylococcus aureus*. One difference was seen. In the last stages of the natural infections, conjunctival folds at various joints broke, and the thick, white hemolymph oozed out. The coccus and rod organisms can be transferred to normal animals by needle inoculations, and recovered from the hemocoelic fluid.

Increased M.D.C. figures obtained with injections of glutathione, cysteine, insulin, and other sulfur-containing compounds are interesting in view of Hammett's recent theory that sulfur as sulfhydryl is the stimulating factor governing cell division[10]. Too, since glutathione is a constituent of muscle and blood[11], the rise in karyokinetic cell values following injections of blood, beef extract, peptone, and nutrient broth can also be explained by the same theory. Murray[12], likewise, has demonstrated a high glutathione content in chick embryos. Could results from injections of embryo extract be due to the presence of sulfur contained in glutathione? Hammett has published much data in support of his contention[13], but, so far, has given no extensive explanation as to how the mechanism is stimulating to the mitokinetic process.

Dinitrophenol and thyroxin are drugs which cause increased metabolic activities of animals. The only clue that might explain their action on hemolymph cells may be related to the fact that the toxic products of increased katabolism in some way stimulate the karyokinetc activities.

In summary of the mitotic phases themselves it is seen that duration of the stages is in the following decreasing order: telophase, prophase, anaphase, and metaphase. The telophase is approximately six times as long as the metaphase.

Only 345, or 0.0066 per cent, amitotic cells were observed. Evidently this method of multiplication could be of little use in the maintaining of hemolymph cell populations. Several cases of nuclear division without subsequent cytoplasmic fission were seen. These were probably stages in the formation of polynuclear cells.

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[10] Hammett, Protoplasma, 7:297-322 (1929).

[11] Hunter, Jour. Biol. Chem., 72:133-167 (1926).

[12] Murray, Jour. Gen. Physiol., 9:621-624 (1926).

[13] Hammett, Science, 79:457 (1934).

# SOME FACTORS INFLUENCING THE GROWTH AND RESPIRATION OF RHIZOBIUM[1]

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Allison, Hoover and Burk (2) and Allison and Hoover (1) reported that several different species of the rhizobia were unable to make any appreciable growth in a synthetic (sugar-mineral-nitrate) medium prepared from highly purified materials. The failure to grow in such a medium was attributed to the absence of a factor essential for the respiration and growth of the organisms. This factor was designated "co-enzyme R." The stimulative effects of a wide variety of substances upon the growth of Rhizobium was attributed to the introduction of appreciable quantities of this co-enzyme.

Repetition of much of the work of Allison and Hoover confirmed their results. Repeated attempts to culture various species of the root nodule bacteria through several consecutive transfers in a mineral salts-$KNO_3$-sucrose c.p. medium were unsuccessful. The addition of such materials as an alcoholic extract of commercial cane sugar, or various plant extracts to the medium induced appreciable increases in growth. A study of the influence of the reaction of the medium upon oxygen consumption and growth of the rhizobia showed that the production of changes in pH of the medium by such substances could in no case account for the stimulative effects noted. Further studies indicated that the results of Allison and Hoover were at least partially due to the medium employed rather than to the inherent characteristics of the organisms. It was found that *Rh. meliloti, Rh. trifolii, Rh. leguminosarum* and *Rh. japonicum* were able to maintain growth when continuously cultured in mineral salts-sucrose c.p. media with $NH_4Cl$ or asparagin as sources of nitrogen, but were unable to make any appreciable growth with $KNO_3$ as the nitrogen source. The work of Allyn and Baldwin (3, 4) as well as the results of the present investigation indicate that $KNO_3$ poises bacterial media at a potential so high as to be unfavorable for the nodule bacteria. The addition of iron to these media composed of highly purified materials was found to bring about considerable increases in the growth of several species of Rhizobium as well as of such common soil bacteria as *Azotobacter vinelandii* and *Bacillus subtilis*. The optimum concentration of iron for the growth of *Rh. meliloti* and *Rh. trifolii* was found to be 10 parts per million parts of medium. Ferric chloride promoted greater growth than ferrous sulfate.

The organisms were unable to attain maximum growth rates in any of the media studied which were composed entirely of purified materials. Aqueous extracts of yeast or alfalfa employed as nitrogen sources led to the greatest growth and oxygen utilization of the rhizobia. The addition of such substances as alcoholic extracts of cane sugar or cane

[1] Original thesis submitted June, 1936. Doctoral thesis number 364.

molasses, clear filtrates of old *Az. vinelandii* cultures, aqueous extracts of soils, cysteine, or thioglycollic acid to cultures, of *Rh. trifolii* and *Rh. meliloti* in mineral salts-sucrose c.p. media with $KNO_3$ or $NH_4Cl$ as the source of nitrogen greatly increased their activity. Asparagin and aspartic acid were unable to replace such substances but acted as very readily available sources of nitrogen. The nodule bacteria were able to attack the amino group of asparagin with greater ease than the amid group. The two carboxyl groups of these compounds seemed to increase the availability of the amino group and also seemed to exert an additional stimulative effect upon oxygen utilization and growth.

The mean respiratory quotient of five species of Rhizobium for 24 hours was highly significantly lower in glucose media containing yeast extract as a source of nitrogen than in similar media with either $NaNO_3$ or $NH_4Cl$ as the nitrogen source. The mean quotient in the asparagin glucose medium was significantly lower than in the $NaNO_3$ medium. In media containing no sugar, yeast extract and asparagin brought about a similar lowering of the mean respiratory quotient of the several species of organisms.

All of the various materials which were able to stimulate the growth and oxygen utilization of the rhizobia when added to $KNO_3$ or $NH_4Cl$-sucrose c.p. media also brought about a decrease in the respiratory quotient of *Rh. trifolii* for the first few hours after inoculation. Yeast and alfalfa extracts lowered the respiratory quotient of the organisms over a longer period of time than did the other materials. Since the respiratory quotient is derived from the ratio

$$\frac{CO_2 \text{ produced}}{O_2 \text{ consumed}},$$

any lowering of this value would indicate a change in the physiological activities of the organisms. The increase in oxygen consumption compared to $CO_2$ production, thus, indicates that the substances studied acted as reducing agents with respect to the nodule bacteria.

The similarity in the response of the organisms to the various substances added when compared to the addition of small quantities of cysteine, and the general effect of all of the stimulative materials in lowering the respiratory quotients of the organisms seem to justify the conclusion that one of the important functions of materials used as accessory growth substances for the rhizobia is the provision of an initial hydrogen donator. Such hydrogen donators, presumably, furnish the organisms with a small amount of a readily available initial source of energy which enables them to make adjustments which seem to be necessary when organisms are inoculated into a new medium. Measurements of oxidation-reduction potentials indicated that most of the substances studied reduced the potential of $KNO_3$ sucrose c.p. media.

The results of the present investigation seem to justify the following conclusions:

1. Rhizobia can be continuously cultured in synthetic media containing highly purified sugars without the addition of any complex, unidentified co-enzymes or accessory growth factors.

2. The inability of previous investigators to obtain growth of the legume bacteria in a synthetic $KNO_3$-sucrose c.p. medium may be accounted for in two ways: (1) the medium employed was deficient in iron, and (2) potassium nitrate, in the concentrations used, poised the medium at a potential unfavorable for the respiration and growth of the organisms.

3. One of the important functions of materials used as accessory growth substances for Rhizobium seems to be to provide an initial hydrogen donator. The role of a hydrogen donator for the nodule bacteria appears to be at least twofold; (1) it tends to lower the oxidation-reduction potential of the medium, and (2) it furnishes the organisms with a readily available initial source of energy which enables them to make the necessary adjustments for the establishment of favorable growth conditions.

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# FURAN MERCURIALS AND DERIVED TYPES[1]

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## A. ORIENTATION IN THE FURAN NUCLEUS

In the investigation of the mercury compounds of furan it has been found that the most useful method for determining the position occupied by the mercury-containing group has been the replacement of this group by a halogen. But before this method can be of use, it is necessary to know the position occupied by the halogen in the derived compound. Important compounds for use as reference compounds in orientation studies are so-called 3,5-dibromo-2-furoic acid, 3-bromo-2-furoic acid, 3,5-dichloro-2-furoic, 4,5-dichloro-2-furoic acid and 3-chloro-2-furoic acid[2]. The structures of these compounds as assigned by Hill and coworkers are wrong. The true structures of the compounds are, respectively: 4,5-dibromo-2-furoic acid, 4-bromo-2-furoic acid, 4,5-dichloro-2-furoic acid, 3,5-dichloro-2-furoic acid, and 4-chloro-2-furoic acid. These structures have been proved to be correct in the following manner: 4-Bromo-2-furoic acid (Hill's 3-bromo-) which may be derived by reduction of one halogen atom of 4,5-dibromo-2-furoic acid (Hill's 3,5-dibromo-) was heated with cuprous cyanide, potassium cyanide, and water in a sealed tube at about 200° C. for several hours[3]. The product was 2,4-furandicarboxylic acid. It was identified by the melting points and mixed melting points of the free acid and the dimethyl ester. Significant yields were obtained and the structure of the end product is known. 4,5-Dibromo-2-furoic acid was decarboxylated to form 2,3-dibromofuran (b.p. 160.5°-162.5° C., $D_{26}^{4}$ 2.117, $n^{25}_{D}$ 1.5430). The halogen atoms of this compound were replaced in the same manner as in the case of 4-bromo-2-furoic acid. The product was 2,3-furandicarboxylic acid, the structure of which is known. It was identified by the melting points and mixed melting points of the free acid and the dimethyl ester.

By the same general method the chlorine atom of 4-chloro-2-furoic acid (Hill's 3-chloro-) was replaced by a carboxyl group. This compound may be obtained by reduction of 4,5-dichloro-2-furoic acid. Although the yield of 2,4-furandicarboxylic acid (and its dimethyl ester) was small, the starting material was pure and the reaction is reliable. Much supporting evidence exists.

The structures of the various monohalogenocrotonolactones obtained from these compounds by Hill and Cornelison[4] are probably wrong. All $\alpha$-halogenocrotonolactones are probably $\beta$-halogenocrotonolactones and vice versa. Support for this theory is found in the fact that the melting

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[1] Original thesis submitted March, 1936. Doctoral thesis number 361.

[2] Hill and Sanger, *Proc. Am. Acad. Arts Sci.*, **21**, 135 (1885); Hill and Jackson, *Proc. Am. Acad. Arts Sci.*, **24**, 320 (1889).

[3] Rosenmund and Struck, *Ber.*, **52B**, 1749 (1919).

[4] Hill and Cornelison, *Am. Chem. J.*, **16**, 188 (1894).

point of the so-called $\alpha$-phenylamidocrotonolactones obtained by Hill and Cornelison by the action of aniline on a so-called $\alpha$-halogenocrotonolactone is nearly that of the $\beta$-phenylamidocrotonolactone obtained from aniline and tetronic acid by Wolff and Schimpff[5]. The general mechanisms for the transformation of the halogenated furan compounds into the halogenocrotonolactones, as postulated by Hill and Cornelison can be employed when the correct structures for the furan compounds and the probable structures for the crotonolactones are substituted for those used by these authors.

The half amide of 2,3-furandicarboxylic acid (m.p. 288°-293° C.) was obtained in small amount when 2,3-dibromofuran was treated with cuprous cyanide, potassium cyanide, and water, as has been described. Whether the amido group is in the 2-position or the 3-position is not definitely known.

3,5-Dichloro-2-furoic acid was reduced to 3-chloro-2-furoic acid (m.p. 153.5°-154.5° C.).

## B. FURAN MERCURIALS AND DERIVED TYPES

In the chemistry of furan, compounds having mercury attached to the nucleus are of both theoretical and practical importance. The ease with which furan has been mercurated is a factor tending toward belief that furan has superaromatic properties[2]. In a practical way mercurials have been found to be excellent derivatives for the identification and stabilization of some furan compounds.

Ethyl 2-methyl-4,5-dichloromercuri-3-furoate was prepared by refluxing ethyl 2-methyl-3-furoate with a buffered solution of mercuric chloride. By replacement of the chloromercuric groups with bromine, followed by hydrolysis, there was obtained 2-methyl-4,5-dibromo-3-furoic acid (m.p. 186°-189° C.) By oxidation of the methyl group 4,5-dibromo-2,3-furandicarboxylic acid (m.p. 242°-243° C.) was obtained. By substitution of one hydrogen of the methyl group by bromine, followed by hydrolysis, there was produced 2-hydroxymethyl-4,5-dibromo-3-furoic acid (m.p. 195°-198° C.); acetate (m.p. 143°-146° C.). By replacement of the $\alpha$-bromine atom of 2,3-dibromofuran there is derived 2-nitro-3-bromofuran (m.p. 74.5°-76° C.), short, thick needles. By means of the general method of Gilman and Wright[6] there were derived from the corresponding carboxylic acids 4,5-dichloro-2-chloromercurifuran (m.p. 182°-182.5° C.) and 3,5-dichloro-2-chloromercurifuran (m.p. 123°-124° C.). Methyl 5-bromo-2-furoate was mercurated by fusion with mercuric acetate and converted to the chloromercuri compound. The resulting methyl 4-chloromercuri-5-bromo-2-furoate (m.p. 234°-235° C.) was converted to known 4,5-dibromo-2-furoic acid by treatment with bromine followed by hydrolysis. No ketone was obtained when the mercurial was treated with ketene. Acetyl chloride under pressure and at elevated temperature split the mercurial and at the same time replaced the bromine atom with a chlorine atom. The final product was methyl 5-chloro-2-furoate (m.p. 40°-42° C.), which was further identified by hydrolysis to the acid.

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[5] Wolff and Schimpff, Ann., 315, 151 (1901).

[6] Gilman and Wright, *J. Am. Chem. Soc.*, 55, 3302 (1933).

The same result was achieved when methyl 5-bromo-2-furoate was heated in a sealed tube with mercuric chloride and acetyl chloride. Occasionally some hydrolysis to 5-chloro-2-furoic acid took place during the reaction. The reaction did not succeed with methyl *p*-bromobenzoate. When methyl 4,5-dibromo-2-furoate was treated with acetyl chloride and mercuric chloride in the same manner, what was probably 4-bromo-5-chloro-2-furoic acid (m.p. 150°-152° C.) was obtained upon hydrolysis. Methyl 4-iodo-5-bromo-2-furoate (m.p. 69°-69.5° C.) was obtained when the chloromercuri group of methyl 4-chloromercuri-5-bromo-2-furoate was replaced by iodine.

Methyl 4-chloromercuri-5-chloro-2-furoate (m.p. 215°-217° C.) was prepared from methyl 5-chloro-2-furoate in the same manner as was employed with the bromine analog. By treatment of this mercurial with bromine, followed by hydrolysis, what was probably 4-bromo-5-chloro-2-furoic acid (see above) was produced.

The results of mercuration of furan compounds indicate that mercuration proceeds in the $\beta$-position in accordance with the rules governing orientation in the benzene nucleus. In a furan compound having a *m*-orienting group in the 2-position and an o-, *p*-orienting group in the 5-position, the 4-position is apparently assumed by an entering mercury-containing group.

# I. THE PRODUCTION OF PAPER FROM CEREAL STRAWS

# II. THE UTILIZATION OF AGRICULTURAL WASTES FOR PRODUCTION OF MISCELLANEOUS FABRICATED MATERIALS[1]

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## I. THE PRODUCTION OF PAPER FROM CEREAL STRAWS

There are in excess of 150,000,000 tons of cornstalks and 70,000,000 tons of cereal straws produced annually in the United States. These materials have an approximate general analysis of 35 to 45 per cent cellulose, resembling wood and cotton cellulose; 15 to 25 per cent pentosan, a source of xylose, furfural, and moulded plastics; and 25 to 35 per cent lignin, a material resembling protein in reaction. Laboratory and commercial trials demonstrated that a satisfactory cellulose for paper and rayon could be made from this source with less drastic and more economical processing than from wood.

This thesis covers the manufacture of paper from cereal straws and cornstalks by the kraft, soda, and other processes, using commercial paper manufacturing methods modified to the extent required by the physical character of these materials. The studies were conducted at the U. S. Bureau of Standards, and are included in the projects of the Agricultural By-Products Laboratory, U. S. Department of Agriculture, at Ames, Iowa. These government bureaus cooperated with the Iowa Engineering Experiment Station.

### EXPERIMENTAL

At the conclusion of the first two years' work on paper, in which the digestion of wheat, oat, and rye straws by the kraft process was studied, a process was outlined and calculations made for the design of a commercial paper mill. Equipment for continuous digestion had the advantage of about 25 per cent reduction in digester capacity and increased thermal efficiency.

The next phase of the paper research was the production of paper from the cortex of the cornstalk The first part duplicated the procedure on cereal straws in which the optimum range of conditions, for the dual digestion with water and chemical, were determined. A study was also made on the repeated use of single batches of water digestion liquor and chemical digestion liquor, to concentrate the extracted material for practical utilization or chemical recovery.

The second part of the cortex studies was the semi-commercial digesion of larger batches of pulp for paper machine runs at the Bureau of Standards in Washington, D. C. The digestions were made in a three-foot spherical rotary digester using direct steam, whereas the laboratory scale

[1] Original thesis submitted July, 1935. Doctoral thesis number 328.

studies were made with indirect steam. Difficulties were experienced with variable dilution from the direct steam and the variable quantity of bone-dry fiber. The dilution difficulties were overcome by a study of steam consumption and radiation. Difficulties with the determination of the quantity of bone-dry fiber were overcome by establishing an emperical relation between the measured cortex displacement of water in the digester and its bone-dry fiber content. It was found that the degree of digestion and refining, and the cleanliness of the pulp, could be judged from the examination of hand sheets under moderate microscope magnification by transmitted light.

The cortex fiber of cornstalks hydrates, or gelatinizes, abruptly in contrast to wood fiber. This characteristic makes cornstalks particularly adaptable to glassine papers. Laboratory scale studies on hydrating cortex from soda digestions were made.

### CONCLUSIONS

1. Paper, varying from wrapping paper to a good grade of bond and glassine papers, can be made from cereal straws and cornstalk cortex, by a water digestion, modified kraft or soda cook, combined beating and bleaching, and cleaning by screening and centrifining.
2. The strength of the paper varies from strongest with oat straw, through barley and wheat, to the weakest with rye straw. Bleachability varies in the reverse order.
3. The calculations for a proposed paper mill indicate the advantage of the re-use of the digesting liquors and of a continuous digester.
4. In using a rotary digester, digestion can be controlled from steam consumption data and from a displacement measurement of the wet fiber.
5. The quality of the hand sheets was controlled by the microscope, using moderate magnification and transmitted light.

## II UTILIZATION OF AGRICULTURAL WASTES FOR PRODUCTION OF MISCELLANEOUS FABRICATED MATERIALS

In the course of experimental studies on agricultural wastes, by the U. S. Bureau of Standards and the Iowa Engineering Experiment Station at Ames, Iowa, the manufacture of diverse products from these materials was studied for practical outlets for substantial quantities of these wastes. Some of these products were new and required inventive faculty, and all the products required adaptation to the distinct characteristics of the agricultural wastes.

This thesis covers phases of the development of several such products.

### EXPERIMENTAL

Cornstalks, which possess two types of fibers with widely different physical characteristics, had previously been separated into two fractions by both wet and dry processes. The separation by each method was studied and these separations were distinctly improved and adapted to better commercial processing. The best wet procedure was found to be shredding the stalks, scrubbing with four or five changes of water, drain-

ing, treatment in an attrition mill, floating the pith and fine fiber up through a screen by means of an upward flow of water, and a final continuous flotation of the pith. A dry attrition treatment was also developed which was followed by air separation, either over an inclined baffle or in a centrifugal air separator.

A process for pressing and moulding complicated shapes, such as window sashes and fiber pipes, as unit structures was developed. The material was waterproofed to a high degree, the products conditioned with moist heat to prevent warping, and tested to demonstrate their strength as unit structures.

A method of solvent sizing pressed fiber products by impregnating the dry fiber with a solution of paraffin or wax in carbon tetrachloride, gasoline, or other solvents, and evaporating the solvent, was developed. The degree of waterproofing was tested by both the standard and special deflection and immersion test.

A method was perfected of determining the moisture content of pressboard in the hydraulic press by measuring the electrical conductivity.

The conductometric method of determining end points in volumetric titrations was applied to the spent liquors from paper making, using practical equipment and simplified procedure. The test was also applied to the titration of pyroligneous acid and acid distillate from the destructive distillation of agricultural wastes.

The precipitation of organic matter from the spent digesting liquors from paper making was studied using acids and soluble salts of Ca, Br, and Sr. The organic material precipitated readily as a heavy filterable floc. The filtrate was clear, the color depending on the precipitating agent. This suggested a procedure whereby the precipitate could be pressed, dried, and the filtrate causticized for further digestions.

## CONCLUSIONS

1. Cornstalk cortex and pith can be divided by wet, moist, or dry attrition treatment, and either water flotation or air separation, with commercial equipment. The cortex is satisfactory for cellulose uses. The pith has a higher thermal insulation value than cork.
2. Complicated forms of cornstalk fiber products can be pressed and moulded as unit structures. These can be cheaply made and excel similar wood products in strength.
3. Cornstalk pressboard is made very moisture resistant by solvent sizing. A deflection and immersion procedure was developed as a test for waterproofing.
4. The change in electrical conductivity can be used to determine the moisture in pressboard and this method is suited to research and technical control. It also shows defects in press design.
5. Conductometric titration can be applied to advantage in the analysis of spent liquor from pulp digesions.

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